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ANTEMORTEM AND POSTMORTEM DRUG LEVELS AND HUMAN PERFORMANCE

ANTEMORTEM AN-D POSTMORTEM DRUG LEVELS AND HUMAN PERFORMANCE

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ANTEMORTEM AND POSTMORTEM DRUG LEVELS AND HUMAN PERFORMANCE

SPECIFIC AIMS AND OBJECTIVES

The mission of the Federal Aviation Administration (FAA), Civil Aeromedical Institute (CAMI), Toxicology and Accident Research Laboratory, Forensic Toxicology Research Section, includes (1) analyzing specimens from fatal aircraft accident victims for the presence of therapeutic, sub-therapeutic and overdose levels of drugs, (2) assisting the FAA and National Transportation Safety Board (NTSB) in determining if the drugs found (or associated diseases for which the drugs are given) may be a contributing factor in accidents, and (3) providing consultation and court testimony to government attorneys in matters being litigated by the US government. The "FAA Research, Engineering and Development Authorization Act of 1990" authorized appropriations for aviation research, engineering and development. This project addressed the areas of human factors and alcohol/drug research detailed in this act. In conducting this project, the University of Oklahoma facilities and personnel were of assistance in providing supportive research and data in the areas of analytical methods development and studies related to the effects of drugs on personnel in the aircraft industry.

Of 379 pilots analyzed in 1991, 6% involved controlled drug substances(CDS)-Schedules I and II), 1% involved CDS (Schedules III-IV), 6% involved prescription drugs, 11% involved over the counter medications and 8% involved alcohol. In one recent review of 87 pilots certified to fly with antihypertensives, 40% had a diuretic in their single or multiple treatment regimens. The dangers associated with concurrent administration of medications (prescription and nonprescription) and flying need to be more thoroughly documented, especially as it involves human performance factors.

The primary benzodiazepine drug prescribed in 1991 was alprazolam, which has a therapeutic range from 6 ng/mL to 17 ng/mL. With such low therapeutic levels, these drugs are difficult to detect and are below the detectable limits of much available technology, consequently, many cases of impaired performance may be occurring but not reported as being due to these drugs.

This overall project was originally to be divided into three phases for each group of three drugs to be studied annually. Phase I was to include the development of automated analytical screening methods for the drugs and the determination of antemortem and postmortem levels of the drugs in an animal model; Phase II was to include Performance Testing and correlation with antemortem levels of the drugs; Phase III was to include a study of the high altitude effects on the pharmacokinetics of the drugs in humans.

Only Phase I of the first group of three drugs was included in this project for the drugs

atenolol, alprazolam and terfenadine. Phases II and III were designed for the second and subsequent years according to Table 1. Three additional drugs were to be added each year so that after three years, the studies involving the first set of three drugs would be complete and a set of three additional drugs were to be completed each subsequent year.

Specifically, Phase I involved:

1. developing methodology for the identification of atenolol, alprazolam and terfenadine.
2. identifying and quantitating atenolol, alprazolam and terfenadine levels in biological fluids and tissues using high performance liquid chromatography (HPLC).
3. the study of the postmortem redistribution of atenolol, alprazolam and terfenadine in blood and other tissues, using rats as a model,
4. determining if there is a significant difference between the concentration in antemortem and postmortem brain, blood and other tissues, and
5. determining whether postmortem blood concentrations or other tissue concentrations could be used as an indicator for accurate interpretation of atenolol, alprazolam and terfenadine levels.

BACKGROUND AND SIGNIFICANCE

The accurate interpretation of drug levels on human performance in postmortem blood and tissues has been a concern for most forensic toxicologists.[1] It has been reported that drug concentrations in blood specimens from different areas of the body may increase or decrease depending on the nature of the drug during the postmortem interval prior to autopsy. [2,3] The changing of drug levels after death has led to the investigation of postmortem redistribution of various drugs. [3] The actual relationship between the levels of drugs in postmortem blood and the various body tissues has not been established and there has been limited information published in the scientific literature. There is concern that postmortem blood and tissue specimens do not actually reflect the antemortem levels of drugs. Consequently, when projects are accomplished using postmortem levels, there is no assurance that the levels have not increased or decreased depending on the nature of the drug and the environment. This redistribution may be due to passive diffusion, pH changes, hemodynamic changes, enzymatic changes, and putrefaction changes.

In addition to actual blood/tissue changes, drug ratios between blood and various tissues can be calculated, i.e., liver:blood, brain:blood, kidney:blood, heart:blood. This may be important if a drug might be hepatotoxic, neurotoxic, renal toxic or cardiotoxic. Not only drugs, but drug metabolites and their ratios can be of importance. An important factor in the determination of drugs in blood and tissues is the actual time of autopsy after death.

It is important to develop a suitable animal model, or models, to work with and to include a wide array of drug substances in addition to recreational/social drugs and drugs of abuse. Drug classes should include beta blockers, ACE inhibitors, antihypertensive agents, antidepressants, antianxiety drugs, antihistamines, analgesics and others.

It is vital that this be studied and that relationships be established identifying:

1. the redistribution of drugs and their metabolites in postmortem situations of drugs/drug substances that are used by pilots and other flight personnel,
2. the levels of drugs and their metabolites in blood and various body tissues at various times postmortem,
3. whether or not there is a significant difference in the concentrations of drugs/drug substances antemortem and postmortem,
4. whether postmortem blood or tissue concentrations or blood:tissue ratios can be used as an indicator for accurate interpretation of drug &/or drug metabolite levels in antemortem blood or tissue levels.

The importance of this research is not limited to accidental deaths or suicides, but is of vital importance in determining actual drug concentrations in the event of automobile, rail and aviation accidents. This information is important not only for the accurate determination of causative factors in airline/railroad accidents, but also to protect the reputation of the airline personnel involved. In addition, it is especially important to begin relating drug levels of these drugs to performance factors of the operators of the vehicles, etc.

Detailed human case data has been reported to illustrate the dramatic extent of the phenomenon of post-mortem drug redistribution.[4] The data suggests that there is a postmortem diffusion of drugs along a concentration gradient, from sites of high concentration in solid organs, into the blood with resultant artifactual elevation of drug levels in blood. Highest drug levels were found in central vessels such as pulmonary arteries and veins, and lowest levels were found in peripheral vessels such as subclavian and femoral veins. This study involved doxepin, desmethyldoxepin, amobarbital, secobarbital, pentobarbital, clomipramine, desmethylclomipramine, flurazepam, imipramine and desipramine. The authors concluded that the phenomenon creates major difficulties in interpretation and undermines the reference value of data bases where the site of origin of postmortem blood samples is unknown.

In another study,[5] it was found that heart blood-drug concentrations were, in general, significantly higher than those of peripheral specimens. As a result of this phenomenon, the analysis of peripheral blood specimens and solid tissues is often necessary before a definitive interpretation of postmortem toxicological analyses is possible. They discuss the site and time dependence that is observed for postmortem blood-drug concentrations.

The importance of related factors, including left ventricular postmortem contraction, the arterial vascular bed, diffusion processes connected to the physicochemical characteristics of the substances, and the anatomical distribution of the vessels has been demonstrated and discussed.[6]

Postmortem redistribution of digoxin has been demonstrated to take place.[7] When the serum drug concentrations are within the therapeutic or low toxic range, digoxin may reenter the blood. High antemortem serum concentrations of digoxin may prevent such passive redistribution. Therefore, antemortem digoxin intoxication cannot be reliably inferred on the basis

of high postmortem levels of the drug. Digoxin intoxication can be ruled out when postmortem serum drug concentrations remain within the therapeutic range.

Postmortem diffusion of aminohippuric acid to the urine has been observed. [8] Postmortem ethanol continues to be a problem in the interpretation of toxicology results.

A postmortem redistribution study was done involving patients receiving cimetidine therapy. [9, 10] Analysis of postmortem tissues and fluids led the authors to conclude that the tissue:serum ratio of cimetidine decreased with time after death. They also concluded that the time of the autopsy after death is also important in determining the concentration of cimetidine in serum, cerebrospinal fluid and other tissues.

In one study, 29 literature overdose cases and 8 non-overdose literature cases were compared. The results indicate significant postmortem redistribution of chloroquine. The results also indicate that using a liver concentration of 150 mg/kg as a cutoff between overdose and non-overdose concentrations properly identified 30 of the 34 published cases containing liver chloroquine and 19 of the 20 presented cases. [11]

Postmortem changes in sulfide concentrations in body tissues have been examined in rats exposed to hydrogen sulfide and in nonexposed rats and humans. [12] Sulfide concentrations in the blood, liver and kidneys of rats increased in both the exposed and nonexposed groups, depending on the lapse of time after death. On the other hand, the lung, brain and muscle showed little or no change in sulfide concentration with elapse of time after death. The data obtained from human tissues were almost the same as those for rats, except data for blood, in which no or little increase of sulfide was observed.

Other studies discuss the site dependent variability of postmortem blood concentrations for selected drugs [8, 13, 14] and certain biochemical changes, e.g. studies reporting differences in canine vitreous humor, [15] cerebrospinal fluid [16] and blood. [17]

Studies have also been performed concerning the postmortem stability of benzodiazepines in blood and tissues. Diazepam, flurazepam and N-1-desalkylflurazepam were demonstrated to be stable when stored in blood at room temperature while chlordiazepoxide, norchlordiazepoxide and nordiazepam were found to be unstable under similar storage conditions. [18]

Three drugs have been selected for this study because of their widespread use in the U.S. population and the age group with which we are concerned. These drugs include atenolol, alprazolam and terfenadine, drugs which are very widely used.

STUDY DRUGS

Atenolol (Tenormin)

Pharmacology: Atenolol is a β_1-selective adrenergic blocking agent that has been approved by the FAA for use by pilots. It has pharmacologic actions similar to those of other β-adrenergic blocking agents. Its principal physiologic action is to competitively block adrenergic stimulation of β-adrenergic receptors within the myocardium and within vascular smooth muscle.

Pharmacokinetics: Atenolol is rapidly but incompletely absorbed from the GI tract. Only about 50-60% of an oral dose of atenolol is absorbed. In healthy adults, peak plasma concentrations of 1-2 μg/mL are achieved 2-4 hours after oral administration of a single 200-mg dose. The effect of atenolol on heart rate usually has an onset of 1 hour, peaks at 2-4 hours, and persists for 24 hours following oral administration of the drug. Atenolol's effect on heart rate, but not on blood pressure, correlates linearly with plasma atenolol concentrations of 0.02-200 μg/mL.

Atenolol is well distributed into most tissues and fluids except brain and CSF. Unlike propranolol, only a small portion of atenolol is apparently distributed into the CNS. Approximately 5-15% of atenolol is bound to plasma protein. In patients with normal renal function, atenolol has a plasma half-life ($t_{1/2}$) of 6-7 hours. Approximately 40-50% of an oral dose of the drug is excreted in urine unchanged. The remainder is excreted unchanged in feces, principally as unabsorbed drug.

Uses: Atenolol is used in the management of hypertension. The drug has been used as monotherapy or in combination with other classes of antihypertensive agents. Atenolol's efficacy in hypertensive patients is similar to that of other β-adrenergic blocking agents. It is also used for the management of chronic stable angina pectoris and appears to be as effective for this indication as are other β-adrenergic blocking agents. The drug is used orally and intravenously to reduce the risk of cardiovascular mortality in patients who have had a definite or suspected myocardial infarction and are hemodynamically stable.

Potentially serious adverse cardiovascular effects of atenolol include bradycardia, which occurs in 3% of patients; profound hypotension; second- or third-degree atrioventricular block; and precipitation of severe congestive heart failure, which is more likely to occur in patients with preexisting left ventricular dysfunction. Adverse CNS effects of atenolol include dizziness, fatigue, and mental depression. Lethargy, drowsiness, unusual dreams, lightheadedness and vertigo usually occur in less than 3% of patients. Adverse GI reactions include diarrhea and nausea, which reportedly occur in 2-4% of patients receiving atenolol.[19]

Physicochemical: Atenolol is a white, crystalline powder with a slightly bitter taste and an aqueous solubility of 26.5 mg/mL. Its solubility in other solvents is:

Solvent	Solubility (*USP terminology)

Methanol	Freely soluble
Acetic acid	Soluble
Dimethylsulfoxide	Soluble
96% Ethanol	Sparingly soluble
Isopropanol	Slightly soluble
Acetone	Very slightly soluble
Dioxane	Very slightly soluble
Acetonitrile	Insoluble
Ethyl acetate	Insoluble
Chloroform	Insoluble

It melts between 150°C and 152°C and has a low partition coefficient for n-octanol-phosphate buffer (0.008 at pH 7.0 and 0.052 at pH 8.0).

Analytical: Analytical methods developed include infrared, ultraviolet, proton magnetic resonance, nuclear magnetic resonance, and mass spectroscopy.[20] Chromatographic methods include thin-layer, high performance thin-layer, gas-liquid and high pressure liquid chromatography. [21-25]

One study involving atenolol was conducted to evaluate the effects of orthostatic, altitude and pharmacologic stresses upon civil aviation-specific performance.[26] The results demonstrated an impaired performance on a computerized cognitive battery during lower body negative pressure stress at altitude. The data implicated the synergistic deleterious effects of beta-blockade and altitude in the potentiation of intolerance to orthostatic stress, especially in personnel of unpressurized aircraft who are being treated with beta-blocking drugs for hypertension. It was also found that there was no common clinical parameter to predict this intolerance, but that only formal stress testing was able to uncover the orthostatic-prone individuals.

Alprazolam (Xanax)

Pharmacology: Alprazolam is a benzodiazepine, shares the actions of other drugs in this class and is used for the management of anxiety disorders or for the short-term relief of symptoms of anxiety or anxiety associated with depressive symptoms. It is one of the top prescribed drugs in the US.

Drugs that affect the CNS may have additive CNS effects when used concomitantly with, or during the period of recovery from, alprazolam. In patients who have received prolonged alprazolam therapy, abrupt discontinuance of the drug should be avoided since manifestations of withdrawal may be precipitated.[27]

Pharmacokinetics: Alprazolam is generally well absorbed from the GI tract and it is widely distributed into body tissues and across the blood-brain barrier. It is also highly bound to plasma proteins. It has a half life of about 12-15 hours and has no significant active metabolite.

Uses: Alprazolam is used for, among other things, the preoperative relief of anxiety, for the management of agitation associated with acute alcohol withdrawal, for the management of anxiety disorders and for the short-term relief of symptoms of anxiety.

Physicochemical: Alprazolam occurs as a white to off-white crystalline powder. Its solubility in various solvents is:

Solvent	Solubility (mg/mL)
Alcohol	14
Water	0.08
Propylene glycol	16
Chloroform	3
Ethyl acetate	30

The drug has pKa values of 1.3 and 11.5 and melts between 167-170°C.

Analytical: Analytical methods include infrared[28], nuclear magnetic resonance[29], ultraviolet [30-33] and mass spectroscopy.[34] Also, colorimetric, polarographic, titrimetric analyses have been reported along with thin-layer, gas and high-performance liquid chromatographic methods.[36-39]

Terfenadine (Seldane)

Pharmacology: Terfenadine is a butyrophenone-derivative antihistamine that is structurally unrelated to other currently available antihistamines. It, too, has been approved for use by pilots. Terfenadine is a specific, selective, histamine H_1-receptor antagonist. The pharmacology of terfenadine resembles that of other currently available antihistamines; however, the overall pharmacologic profile of terfenadine differs from that of these other drugs. Unlike most other currently available antihistamines, terfenadine appears to have a dual effect on histamine H_1-receptors. Experimental evidence indicates that the drug exhibits a specific and selective antagonism of histamine H_1-receptors and that the drug slowly binds to the H_1-receptor and forms a stable complex from which it subsequently dissociates. These findings suggest that the prolonged and generally irreversible nature of terfenadine's antagonism of histamine results principally from the drug's slow dissociation from the H_1-receptors.

Unlike many other currently available antihistamines, terfenadine does not possess appreciable anticholinergic or antiserotonergic effects at usual antihistaminic doses in pharmacologic studies. However, in clinical trials, there was no difference in the frequency of anticholinergic-like effects (e.g., dryness of the nose, mouth, throat, and/or lips) observed with terfenadine or other antihistamines.

Pharmacokinetics: Although at least 70% of an oral dose of terfenadine is rapidly absorbed from the GI tract following oral administration, the drug undergoes extensive first-pass

metabolism in the liver and/or GI tract, with only minimal or undetectable amounts of an orally administered dose of the drug appearing to reach systemic unchanged in healthy individuals.

Following oral administration of a single 60-mg terfenadine dose, peak plasma concentrations of the drug occur at about 1-2 hours. In one study in healthy adults, mean peak plasma terfenadine concentrations of 1.5 or 4.5 ng/mL, respectively, occurred at about 1 hour following oral administration of a single 60 or 180 mg dose of the drug. In one study following oral administration of a single 60 mg dose of terfenadine in healthy adults, mean peak plasma carboxylic acid derivative concentrations of 263 ng/mL were attained in about 2.5 hours; unchanged plasma terfenadine concentrations were undetectable in these individuals. Although a linear correlation between dosage and peak plasma concentration reportedly exists following oral administration of single terfenadine doses of 60 or 180 mg, the area under the plasma concentration-time curve for terfenadine reportedly increases nonlinearly with dose.

Following oral administration, the antihistaminic effect of the drug is apparent within 1-2 hours, maximal within 3-6 hours and persists for 12 hours or longer.

Distribution of terfenadine and its metabolites into human body tissues and fluids has not been determined. Following oral administration of terfenadine in animals, the drug and its metabolites are distributed mainly into the liver, lungs, spleen, GI tract, and bile, with lower concentrations being distributed into kidneys, heart and blood. Terfenadine does not appear to appreciably cross the blood-brain barrier in animals. In vitro, terfenadine and the carboxylic acid metabolite are approximately 97 and 70% bound, respectively, to plasma proteins.

It appears that only minimal or undetectable amounts of an orally administered dose of the drug appear to reach systemic circulation unchanged in healthy individuals. Plasma concentrations of the carboxylic acid metabolite generally have been reported to decline in a biphasic manner; however, there is preliminary evidence that the metabolite may exhibit triphasic elimination with a prolonged terminal phase (over 14 hours) following administration of a high dose of terfenadine. In adults with normal renal and hepatic function, the initial elimination half-life is about 6 hours. Following oral administration of multiple doses of terfenadine, the steady-state plasma elimination half-life of the carboxylic metabolite was reported to be 8.5 hours.

Approximately 60 and 40% of an oral dose of the drug is excreted in feces and urine, respectively, within 24-48 hours, principally as metabolites. In healthy individuals, about 16% of a single oral dose of terfenadine is excreted in urine as the carboxylic acid derivative, 12% as the piperidine carbinol derivative, and 12% as minor unidentified metabolites; about 30% of a single dose of terfenadine is excreted in feces as the carboxylic acid metabolite, 1.2% as unchanged drug, and about 28.8% as minor unidentified metabolites.

Terfenadine alone or in fixed combination with pseudoephedrine hydrochloride is used to provide symptomatic relief of seasonal allergic rhinitis. It has also been used for the symptomatic treatment of perennial allergic rhinitis, as well as for some other disorders.

Uses: Terfenadine has been used for the treatment of various allergic dermatologic conditions associated with histamine release, the treatment of pruritus associated with chronic obstructive liver disease, and it has been used to increase bladder capacity in a limited number of patients with neurogenic bladder and overactive detrusor function.

The most frequent adverse effects reported with terfenadine therapy are sedation and headache, which occur in about 5-16% of patients receiving the drug. Other less frequent adverse nervous system effects include dizziness, nervousness and weakness. Adverse GI effects reportedly occur in about 5-8% of patients and include abdominal distress, nausea, vomiting and a change in bowel habits. Dry mouth, nose, throat and/or lips; cough; sore throat; and epistaxis occur in less than 5% of patients receiving this drug. severe cardiac effects, including arrhythmias have been reported rarely in patients receiving terfenadine; usually these adverse effects were associated with very high daily dosages.[40]

Physicochemical: Terfenadine occurs as a white to off-white crystalline powder with a very bitter taste. It occurs as three different polymorphs with melting ranges from 149-152°C, 146-148°C and 142-144°C, respectively for polymorphs I, II and III. Its solubility is as follows:

Solvent	Solubility (G/100 mL at 30°C)
Water	0.001
Ethanol	3.780
Methanol	3.750
Hexane	0.034
0.1 M Hydrochloric Acid	0.12
0.1 M Citric acid	0.110
0.1 M Tartaric acid	0.045

The pKa of terfenadine is 10 and it has been characterized by differential scanning calorimetry.[41]

Analytical: Analytical methods include ultraviolet florescent, infrared, mass, and nuclear resonance spectroscopy, elemental analysis and non-aqueous titration. Also, thin layer chromatography[42], high pressure liquid chromatography[43-48], radioimmunoassay[49], C-14 analysis[17] and gas chromatography-mass spectrometry[17] have been reported.[50-52]

PRELIMINARY STUDIES

We have been involved in analytical methods development, pharmacokinetic studies and analytical methods validation/comparison studies for several years.[53-66]

In some of our previous work, the antemortem and postmortem redistribution of amitriptyline (AMI) was studied in rats.[66] The high performance liquid chromatography analytical technique utilized for the analysis was simple, rapid, sensitive, reliable and very specific for the

separation and quantitation of the drug. The efficiency of the liquid:liquid extraction methods using hexane was increased approximately 30% with the addition of 0.5% diethylamine. The extraction and analytical technique was very sensitive for detecting AMI in rat blood and other tissues at a minimum concentration of 10 ng/mL.

We observed a higher postmortem brain:blood ratio suggesting the tendency of AMI to penetrate into the brain, possibly due to AMI's lipophilicity. Postmortem AMI levels in the brain increased with an increase in the postmortem period. This may be due to hydrolysis and putrefaction resulting in the breakdown of macromolecules. There were higher levels of AMI in the liver with an increase in postmortem time which might be related to AMI glucuronide hydrolysis. A high tissue:blood concentration ratio for AMI after death was observed indicating disequilibrium AMI concentrations existing between the blood and various tissues. Other studies have been reported involving postmortem redistribution of the drug in rabbits[67] and one involving the interpretation of deaths involving tricyclic antidepressant drugs based on tissue distribution.[68]

GENERAL PLAN OF WORK

This current study consisted of the development and validation of automated extraction and analytical screening methods and the determination of antemortem and postmortem levels of the selected drugs in various tissues in an animal model. The drugs for this project included atenolol, alprazolam and terfenadine.

This current study involved:

1. Development and optimization of HPLC methods of analysis for atenolol, alprazolam and terfenadine.
2. Development and optimization of extraction procedures, both liquid:liquid and solid phase, for atenolol, alprazolam and terfenadine.
3. Automation of the extraction procedures by writing programs for the Microlab 2200 automated liquid handling system. Once the protocols are prepared and the programs are written and stored in the computer, the analysis for these drugs can be accomplished rather rapidly. This will serve as a rapid sample preparation method for other laboratories for the analysis of these drugs, i.e., the program can be transferred to other laboratories for implementation.
4. Conducting the antemortem and postmortem studies in rats for atenolol, alprazolam and terfenadine.
5. Data collection, evaluation and report preparation.

EXPERIMENTAL

Materials

The materials, manufacturer and lot numbers of the chemicals and reagents used in this study were as follows:

Chemical Name	Manufacturer	Lot Number
Acetonitrile	Fisher Chemical	942505
Alprazolam	USP Reference Standard	NDC #00216-0104-05
Ammonium Phosphate	Sigma Chemicals	80H0086
Atenolol	Sigma Chemicals	111H0429
Boric Acid	Fisher Chemical	850985
Ethanol	Quantum	0290
Ether	Fisher Chemical	943857-36
Ethyl Acetate	Fisher Chemical	872086
Hexane	Fisher Chemicals	741553
Methanol	Fisher Chemical	945271
Metoprolol	Sigma Chemicals	34H0117
Midazolam	Hoffman-LaRoche	820073
Nadolol	Sigma Chemicals	79F1014
Polyethylene Glycol 300	Carbowax 300	UCCB18221
Phosphoric Acid	Fisher Chemical	911428
Propylene Glycol	J.T. Baker	625628
Sodium Citrate	J.T. Baker	C28723
Trichloracetic Acid	Sigma Chemicals	113H02551
TEAA	Fluka	G07268
Terfenadine	Sigma Chemicals	92H0705
Tween 80	Fisher Chemicals	871759

Equipment used in this project was as follows:

N-Evap Analytical Evaporator, Model 112, Assoc. Inc.		
Gelman Filters, 25 mm, 0.45µ		
C18, 5 u 4.6 x 250 mm column	JT Baker	H11084-20
C8, 5 u 4.6 x 250 mm column	Alltech	94029128
CN, 5 u 4.6 x 250 mm	JT Baker	G50084-07
LC-6A HPLC Pump	Shimadzu	278155MS
SIL-9A Autoinjector	Shimadzu	C20273002030
RF-535 Fluorescent Detector	Shimadzu	1261366
SPD-6A UV Detector	Shimadzu	80069S
Polytron Kinematica	Brinkmann Instr.	3389

Microcentrifuge	Fisher	10MUSS
Centrifuge, CU-5000	Damon/IEC	23473953

Methods: General

The overall system developed involved automated sample preparation using a robotic system and/or solid phase or liquid/liquid extraction followed by analysis by high performance liquid chromatography.

Development and optimization of HPLC:

Pure drug reference standards and metabolites were obtained where available. All standards were stored according to the manufacturer's requirements or at 5°C in a desiccator and were allowed to come to room temperature in the desiccator prior to weighing. Standard solutions were prepared in Milli-Q water, except where alternate solvents were required. All mobile phases and buffers were prepared from HPLC grade solvents and Milli-Q water and were filtered through a 0.22μ filter and degassed prior to use.

HPLC:

The linear response of the detectors was established by constructing calibration curves using standard solutions. The recovery of the analytes was determined by comparing the peak areas after direct injection of a standard solution with those obtained from the blood and tissue extracts.

Standard curves were constructed by spiking drug-free blood or tissue homogenates, mixing, extracting the drug from the matrices and analyzing for drug content.

General development and optimization of extraction procedures:

The extraction methods used involved classical liquid:liquid and solid-phase extraction (SPE). SPE makes the preparation stage of sample handling faster, easier and more automatable. The extraction methods were optimized from published methods where available and incorporated the physicochemical information obtained for each drug/metabolite(s) and served as a comparison to the newly developed SPE methods. Initial extraction studies were from aqueous solutions, followed by spiked plasma and tissue homogenates, and then from rats to which the drug has been administered. Sufficient studies were done to achieve good precision with the techniques prior to beginning the antemortem and postmortem studies.

General automation of the extraction procedures:

As sample preparation is usually the most tedious, time-consuming and error-prone, it was automated to the extent feasible in this project. The samples were prepared using SPE with a

Microlab 2200 Automated Liquid Handling System. This unit transfers samples, dilutes samples, adds reagents, performs serial dilutions, solid phase extractions, concentrations and timed reactions. The protocol was developed and then programmed into the Microlab 2200. Numerous runs were accomplished to validate this system and compare it to manual methods. The protocol required the delivery of the sample to the SPE tube, washing, removing unwanted materials, recovering the drug and metabolite(s) of interest, concentration/drying, reconstitution for HPLC or HPCE analysis and placement in the autoinjector vials (through the septum). However, due to the lack of standard pressure control on the Hamilton unit, manual methods were actually used for the postmortem study.

General antemortem and postmortem studies in rats:

Drug Dosages and Their Administration:

The drug dosages were chosen to be below the LD50 dosages given in the literature. The amounts were also dictated by the maximum solubility of each in their respective matrices. Intravenous formulations were not found in the literature for terfenadine and alprazolam. Components of the matrices were chosen based on solubility and lack of side effects to the rats. Doses 1 (6mg/kg), 2 (8 mg/kg) and 3 (10 mg/kg) of terfenadine were dissolved in ethanol:PEG 300:Tween 80(1:1:1) giving concentrations of 6 mg/ml, 8 mg/ml and 10 mg/ml. Doses 1 (30 mg/kg), 2 (25 mg/kg) and 3 (35 mg/kg) of atenolol were dissolved in PEG 300:propylene glycol:water(1:1:1) giving concentrations of 30 mg/ml, 25 mg/ml and 35 mg/ml. Doses 1 (4.8 mg/kg), 2 (7.8 mg/kg) and 3 (10 mg/kg) of alprazolam were dissolved in PEG 300:propylene glycol:water:ethanol(1:1:1:1) giving concentrations of 4.8 mg/ml, 7.8 mg/ml and 10 mg/ml.

Animal Treatment:

Sprague-Dawley rats weighing 180-200 grams were purchased from Sasco. The rats were fed ad libitum and given 12 hours of light and dark each day. The rats were anesthetized with ether before drug injection. Drugs were injected through the tail vein with a volume of the drug formulation yielding the desired dosage for their weight. Three rats were used for each sampling time and each dosage. After administration of the drug, the rats were returned to their cages for one hour before being euthanized with ether. The euthanasia took approximately 5-7 minutes. Postmortem rats were stored in an exhaust hood at room temperature until they were dissected and their hearts, kidneys, brains, livers and blood were sampled at 0, 1, 6, 12, 24 and 48 hours.

Dissection and Sample Treatment:

At the desired sampling time, a vertical cut was made on the rats abdominal region. Heart puncture was performed and blood was put in a pre-weighed Eppendorf centrifuge vial containing 200 µl of anticoagulant (4% sodium citrate). The vial containing the blood and anticoagulant was weighed and the amount of blood determined from the difference. Blood samples were

centrifuged using a Fisher Microcentrifuge at 5000 rpm for 10 minutes before the plasma was removed to a new pre-weighed vial. Plasma was used because whole blood would lyse when frozen, thus giving inaccurate results. The plasma samples were frozen at -70°C until extraction. Hearts, kidneys, livers and brains were then removed, trimmed of excess tissue and blotted on absorbent paper before placing in pre-weighed vials. The vials were weighed again, and the organ weight was determined from the difference. Care was taken to insure that organs from different rats were trimmed to the same extent. The tissues were then homogenized in Milli-Q water to give a concentration of 0.33g of tissue per ml of homogenate. The tissues were homogenized in a Polytron Kinematica homogenizer for approximately 30 seconds at a setting of 8 while the vial was submerged in iced water to prevent heat degradation. All homogenate samples were then stored in -70°C until extraction.

General observations during the dissection and sampling processes:

The amount of blood obtained decreased after 6 hours postmortem. No anticoagulants, such as heparin, were given to the rats in order to minimize drug interactions. After 12 hours, 24 and 48 hours, sampled tissues were considerably softer than those of previous times and they had disagreeable odors. Both could be attributed to the natural decay process.

Extraction Procedures:

The frozen homogenate samples were thawed at room temperature and vortexed vigorously to ensure homogeneity before extraction. Internal standards with retention times not coinciding with the drug, it's metabolites, or other tissue components were chosen.

Atenolol was extracted from the heart, kidney, liver, brain and plasma by the following procedure: 200 µl of homogenate tissue was transferred to an Eppendorf vial containing 100 ng of internal standard, nadolol. 800 µl of 1M trichloroacetic acid (TCA) was then added to precipitate proteins. The vials were vortexed vigorously for approximately 1 minute before sonicating in ice water for 5 minutes. Then they were placed in a 24-position Beckman GS-15 R refrigerated centrifuge and centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean Eppendorf tube and stored at -70°C before analysis by HPLC. The extraction efficiency for this procedure was approximately 100%. Standard curves were prepared from extraction, using the above procedure, of blank tissue homogenates containing concentrations of atenolol ranging from 50-500 ng/mL and 100 ng/mL of nadolol. Interday and intraday samples were prepared with a known concentration of atenolol and 100 ng of nadolol in human plasma and extracted with the above procedure. Five samples from three separate days were prepared and analyzed to validate the system before postmortem sample analysis.

Terfenadine was extracted from the heart, kidney, liver, brain and plasma by the following procedure: 200 µl of the homogenate tissues were transferred to an Eppendorf vial containing 400 ng of internal standard, metoprolol. 800 µl of acetonitrile (ACN) was added to precipitate proteins. The vials were vortexed vigorously for approximately 1 minute before sonicating in ice

water for 5 minutes, then placed in a 24-position Beckman GS-15 R refrigerated centrifuge and centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a clean Eppendorf tube and stored at -70°C before analysis by HPLC. The extraction efficiency for this procedure was approximately 100%. Standard curve samples were prepared from extraction, using the above procedure, of blank tissue homogenates containing concentrations of terfenadine ranging from 50-500 ng/mL and 400 ng of metoprolol. Interday and intraday samples were prepared with a known concentration of atenolol and 400 ng of metoprolol in human plasma and extracted with the above procedure. Five samples on three separate days were prepared and analyzed to validate the system before rat sample analysis.

ACN and TCA were used to precipitate proteins for atenolol and terfenadine and the reason one was not used for both drugs was a function of the HPLC system used for each drug.

Alprazolam was extracted from the heart, kidney, brain and liver by the following procedure: 500 µl of the homogenate kidney, brain and liver tissues and 400 µl of the homogenate heart tissues were transferred to 15 ml polypropylene centrifuge vials containing 500 ng of the internal standard, midazolam. The heart tissue was less than the others because first time extraction of the heart was "dirty" and the amount of tissue left was a little over 400 µl. 500 µl of 1M borate buffer(pH 9) was used to raise the pH of the tissue and then 4 ml of a 1:1 mixture of hexane and ethyl acetate was added. The samples were then vortexed vigorously for approximately 1 minute and centrifuged for 10 minutes at 6000 rpm in a Damon/IEC CU-5000 centrifuge. The supernatant was transferred to a clean tube and evaporated in the N-Evap analytical evaporator using laboratory compressed air (low pressure). The evaporated samples were then reconstituted with 400 µl of mobile phase and filtered with a 25 mm diameter, 0.45 µ Gelman filter before HPLC analysis. The alprazolam plasma extraction procedure was as follows: 250 µl of plasma was transferred to an Eppendorf vial containing 500 ng of internal standard, midazolam. 250 µl of 1M borate buffer(pH 9) was used to raise the pH of the plasma, then 800 µl of ether was added. Samples were then vortexed vigorously for approximately 1 minute and centrifuged for 10 minutes at 5000 rpm in a 24-position Beckman GS-15 R refrigerated centrifuge. The supernatant was transferred to a clean tube and evaporated in the same manner as the other tissues. The evaporated samples were then reconstituted with 300 µl of mobile phase and filtered with 0.45 µ Gelman filter before HPLC analysis. The extraction efficiency for this procedure was approximately 80%. Standard curve samples were prepared from extraction, using the above procedure for each tissue, of blank tissue homogenates containing known concentrations of alprazolam ranging from 25-500 ng/mL of alprazolam and 500 ng/mL of midazolam. Interday and intraday samples were prepared with a known concentration of alprazolam and 500 ng of midazolam in human plasma and extracted with the above procedure. Five samples from three separate days were prepared and analyzed to validate the system before rat sample analysis.

HPLC System:

Atenolol was analyzed by HPLC with fluorometric detection. The system consisted of a Shimadzu LC-6A pump, a SIL-9A autoinjector, a RF-535 fluorescent detector with a CR601 integrator. A cyano (CN) 5 μ packing column was used. The mobile phase consisted of 86.65% water, 13.29% ACN and 0.05% o-phosphoric acid at a flow rate of 1ml/min and detection at excitation and emission wavelengths of 220 nm and 300 nm, respectively. 40 μl of brain and 20 μl of heart, kidney, brain and plasma were injected with a mobile phase and a control sample injected after every six samples to check for system consistency.

Terfenadine was analyzed by HPLC with fluorometric detection. The system consisted of a Shimadzu LC-6A pump, a SIL-9A autoinjector, a RF-535 fluorescent detector with a CR601 integrator. A C_8 5 μ packing column was used. Mobile phase consisted of 6.25% 0.1M TEAA at pH 5, 33% ACN and 60.75% MeOH with a 1ml/min flow rate and detection at excitation and emission wavelengths of 220 nm and 280 nm, respectively. 20 μl of extracted heart, kidney and liver samples were injected. 50 μl of extracted brain and plasma were injected with a mobile phase and a control sample after every six samples to check for system consistency.

Alprazolam was analyzed by HPLC with ultraviolet detection. The system consisted of a Shimadzu LC-6A pump, a SIL-9A autoinjector, a multi-wavelength SPD-6A UV detector with a CR601 integrator. A C_{18} 5 μ packing column was used. Mobile phase consisted of 43.5% 0.05M ammonium acetate at pH 6.5, 13% ACN and 43.5% MeOH with a 1ml/min flow rate and a detection wavelength of 240 nm. 20 μl of extracted heart, kidney, brain and liver samples were injected. 50 μl of extracted plasma was injected. A mobile phase and a control sample were injected after every six samples to check for system consistency.

These procedures are summarized in Table 3.

Calculation:

Nanogram per milliliter of the drug was calculated from the standard curves. The calculation of drug concentrations per gram of the heart, kidney, brain and liver were derived as follows:

(ng of drug/mL of analyte) X (1 mL of analyte/amount of tissue in 1 mL of homogenate) X (1 mL of tissue homogenate / 1 gram of tissue)

For plasma:

(ng of drug/mL of analyte) X [1 mL of analyte/(amount of plasma + anticoagulant] X (plasma + anticoagulant) / 1 mL plasma

RESULTS AND DISCUSSION

Standard Curves:

The standard curve summaries for the HPLC analytical methods for the drugs in various tissues is summarized as follows:

Alprazolam	Heart	$y = 1.931 \text{ X } 10^{-3} x - 0.03720$	$r^2 = 0.982$
	Kidney	$y = 2.769 \text{ X } 10^{-3} x + 0.05230$	$r^2 = 0.993$
	Brain	$y = 1.975 \text{ X } 10^{-3} x + 0.00290$	$r^2 = 0.991$
	Liver	$y = 2.861 \text{ X } 10^{-3} x - 0.03939$	$r^2 = 0.999$
	Plasma	$y = 1.666 \text{ X } 10^{-3} x - 0.13450$	$r^2 = 0.999$
Atenolol	Heart	$y = 5.927 \text{ X } 10^{-3} x - 0.03255$	$r^2 = 0.998$
	Kidney	$y = 5.288 \text{ X } 10^{-3} x + 0.04150$	$r^2 = 0.999$
	Brain	$y = 6.476 \text{ X } 10^{-3} x + 0.03750$	$r^2 = 0.994$
	Liver	$y = 5.375 \text{ X } 10^{-3} x - 0.01350$	$r^2 = 0.999$
	Plasma	$y = 5.407 \text{ X } 10^{-3} x - 0.04160$	$r^2 = 0.999$
Terfenadine	Heart	$y = 9.261 \text{ X } 10^{-3} x - 0.01196$	$r^2 = 0.999$
	Kidney	$y = 4.074 \text{ X } 10^{-3} x + 0.07357$	$r^2 = 0.996$
	Brain	$y = 4.484 \text{ X } 10^{-3} x + 0.03588$	$r^2 = 0.994$
	Liver	$y = 4.621 \text{ X } 10^{-3} x + 0.01617$	$r^2 = 0.989$
	Plasma	$y = 4.785 \text{ X } 10^{-3} x - 0.09250$	$r^2 = 0.998$

As is evident, the correlation coefficients are greater than 0.99 in all but one case, the terfenadine in the liver samples. The intraday and interday validations study results for alprazolam, atenolol and terfenadine are shown in Table 4. The coefficients of variation for alprazolam were in the range of 4%, for atenolol, about 4-5% and for terfenadine, about 3%. This is considered quite good for a study involving biological samples with extraction procedures.

The results of these studies are shown in Figures 1 through 79. A summary of these figures is as follows:

Item	Tissue	Figure Numbers		
		Alpra	Aten	Terf
Standard curve chromatograms	Heart	1	10	19
Standard curve chromatograms	Kidney	2	11	20
Standard curve chromatograms	Brain	3	12	21
Standard curve chromatograms	Liver	4	13	22
Standard curve chromatograms	Plasma	5	14	23
Composite in different tissues		6	15	24
Validation chromatograms		7	16	25

Chromatograms of extracts without drugs		8	17	26
Chromatograms of standards		9	18	27
Chromatogram of postmortem tissue				28
Standard curves using tissues		29	46	63
Data graphs	Plasma	30	47	64
Data graphs	Brain	31	48	65
Data graphs	Heart	32	49	66
Data graphs	Kidney	33	50	67
Data graphs	Liver	34	51	68
Dosing study results	Plasma	35	52	69
Dosing study results	Brain	36	53	70
Dosing study results	Heart	37	54	71
Dosing study results	Kidney	38	55	72
Dosing study results	Liver	39	56	73
Point-to-point plots, dose 1		40	57	74
Second-order plots, dose 1		41	58	75
Point-to-point plots, dose 2		42	59	76
Second-order plots, dose 2		43	60	77
Point-to-point plots, dose 3		44	61	78
Second-order plots, dose 3		45	62	79

Figures 1 through 5 show the results of the standard curve construction from 25 to 500 ng/mL in different tissues. Alprazolam has a retention time for alprazolam of about 10 minutes and for the internal standard, midazolam, of about 20 minutes. As shown on the composite in Figure 6, the chromatographic procedures for the various tissues separated the intact drug and the internal standard quite well. The brain and plasma were among the cleanest injections with the kidney extracts having more peaks than the other tissues. Figure 7 illustrates the consistency of the procedures for the alprazolam HPLC validation studies, showing 6 different injections. The tissues that were extracted containing no alprazolam or internal standard are shown in Figure 8. Figure 9 shows alprazolam 10 ng/mL, 50 ng/mL and α-OH alprazolam at 100 ng/mL. The alprazolam is eluting at about 10 minutes and the α-OH alprazolam at about 8 minutes.

Figures 10 through 14 show the standard curve chromatograms for atenolol, 50 to 500 ng/mL, for the various tissues. Atenolol has a retention time of about 8.7 minutes and the nadolol internal standard of about 11.5 minutes. As shown on the composite in Figure 15, the chromatographic procedures separate the intact drug from the internal standard with the heart and brain tissue providing the cleanest chromatograms. Figure 16 shows five injections for the atenolol HPLC validation studies with the data illustrated in Table 4. The extracts of the tissues without atenolol are shown in Figure 17 and are very clean after the initial peaks. Chromatograms illustrating levels of 10, 20 and 100 ng/mL are shown in Figure 18 along with an internal standard of 50 ng/mL.

Figures 19 through 23 show chromatograms illustrating the construction of the standard

curves for terfenadine, ranging from 50 to 500 ng/mL. Terfenadine has a retention time of about 17 minutes and the internal standard, metoprolol, has a retention time of about 14 minutes. As shown in the composite, Figure 24, all tissues are relatively clean, with plasma being exceptionally well defined. The validation chromatograms, shown in Figure 25, show good reproducibility with the specific data calculated and shown in Table 4. The extracts of the heart, kidney, brain, liver and plasma without the terfenadine or the internal standard are shown in Figure 26. HPLC chromatograms of 50 ng/mL and 500 ng/mL of terfenadine and 400 ng/mL of metoprolol are shown in Figure 27. Figure 28 shows a representative chromatogram of terfenadine in plasma 48 hours postmortem.

Alprazolam Postmortem Studies:

The standard curve graphs for alprazolam in heart, kidney, brain, liver and plasma are shown in Figure 29. A time series plot after administration of three different doses of alprazolam (4.8 mg/kg, 7.8 mg/kg, and 10 mg/kg) show drug levels in the plasma in Figure 30; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The lower doses showed very little change over 48 hours but the higher dose of 10 mg/kg showed a loss of the drug from the plasma during the first 12 hours postmortem.

A time series plot after administration of three different doses of alprazolam (4.8 mg/kg, 7.8 mg/kg, and 10 mg/kg) show drug levels in the brain in Figure 31; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The results here are similar to the plasma, i.e., the lower doses do not show much change over 48 hours but the 10 mg/kg dose shows a decrease in drug concentration in the brain over about 12 hours.

A time series plot after administration of three different doses of alprazolam (4.8 mg/kg, 7.8 mg/kg, and 10 mg/kg) show drug levels in the heart in Figure 32; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. Again, the lowest dose does not show much change after 48 hours, however, the middle dose of 7.8 mg/kg shows an increase in the heart concentration from 24 to 48 hours and the highest dose, 10 mg/kg, shows a decrease up to 24 hours and an increase at the 48 hour mark.

A time series plot after administration of three different doses of alprazolam (4.8 mg/kg, 7.8 mg/kg, and 10 mg/kg) show drug levels in the kidney in Figure 33; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The overall trend for the 4.8 mg/kg dose is no change, with very slight variations in the 7.8 mg/kg dose and a decrease in the 10 mg/kg dose from the initial time to 24 hours postmortem.

A time series plot after administration of three different doses of alprazolam (4.8 mg/kg, 7.8 mg/kg, and 10 mg/kg) show drug levels in the liver in Figure 34; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The results in the liver are similar to those in the heart, i.e., not much change with the lowest dose but a slight increase with the middle dose between 24 and 48 hours and, for the highest dose, a decrease up to about 12

hours followed by a slight increase from 24 to 48 hours.

The composite plasma data for the three separate dosing studies are shown in Figure 35. The overall change for the 4.8 mg/kg dose is from initial plasma values of about 500-800 ng/mL to about 200-400 ng/mL after 48 hours, a slight decrease. For the 7.8 mg/kg dose, the variability was somewhat greater and not much change really being demonstrated here. At the 7.8 mg/kg range, however, an initial plasma concentration of about 1700 ng/mL dropped to about 700 ng/mL after 24 hours and to about 500 ng/mL after 48 hours.

The composite brain data for the three separate dosing studies are shown in Figure 36. The 4.8 mg/kg dosing level showed initial brain levels of about 900 ng/g dropping to about 300 ng/g after 24 hours and rising to about 450 ng/g after 48 hours. The middle dose of 7.8 mg/kg showed a drop from about 1100 ng/g initially to about 700 ng/g after 24 hours rising to about 1150 ng/g after 48 hours. The highest dose of 7.8 mg/kg provided initial doses in the brain of about 3000 ng/g falling to less than 1000 ng/g after 24 hours and rising to about 1300 ng/g after 48 hours.

The composite heart data for the three separate dosing studies is shown in Figure 37. For the lowest dose, this tissue shows an initial decrease after one hour from about 1600 ng/g to about 900 ng/g, then an increase after 6 hours to about 2000 ng/g, then a decrease to about 24 hours to about 1000 ng/g followed by a slight increase to about 1400 ng/g after 48 hours. For the middle dose, the initial concentrations were close, at about 1800 ng/g for about 6 hours, followed by an increase after 12 hours to about 3200 ng/g, then a decrease after 24 hours to about 1000 ng/g, then an increase to about 3600 ng/g after 48 hours. The highest dose, 10 mg/kg, resulted in an initial decrease in one hour from about 5500 ng/g to about 2000 ng/g, an increase to about 4500 ng/g after 6 hours, a decrease to about 1900 ng/g after 12 hours and then a slight increase after 48 hours to about 3000 ng/g.

The composite kidney data for the three separate dosing studies is shown in Figure 38. The lowest dose shows a slight downward trend overall from about 1600 ng/g to about 900 ng/g after 48 hours. The middle dose remains almost unchanged over 48 hours, despite some variability and a slight increase after 6 and 12 hours. The high dose showed only a slight decrease overall with a dip after 24 hours.

The composite liver data for the three separate dosing studies is shown in Figure 39. For the low dose of 4.8 mg/kg, there was a consistent decrease from about 1800 ng/g to about 900 ng/g after 24 hours followed by an increase to about 1700 ng/g after 48 hours. The middle dose of 7.8 mg/kg showed relative consistency for 24 hours followed by an increase after 48 hours, from an initial concentration of about 2200 ng/g to a final value of about 3000 ng/g. The highest dose of 10 mg/kg resulted in very little change over 48 hours. A slight decrease over the first 12 hours and a slight increase over the last 24 hours.

The low dose studies are plotted in a composite tissue plot, point-to-point, in Figure 40

and as a second-order regression in Figure 41. Figure 41 shows the liver concentrations declining up to 24 hours and increasing from 24 to 48 hours. There is a general, slight decline, in the other tissues over the 48 hour time period.

The middle dose studies, 7.8 mg/kg, are plotted in a composite tissue plot, point-to-point, in Figure 42 and as a second-order regression in Figure 43. Figure 43 shows an initial decrease followed by an increase for three tissues; heart, liver and brain. The plasma levels were relatively constant and the kidney levels increased and decreased over time.

The high dose studies for alprazolam are plotted as a composite tissue plot, point-to-point in Figure 44 and as a second-order regression in Figure 45. There is an overall decrease in most tissues, including the kidney, brain and plasma with a decrease followed by an increase for the heart and the liver.

Alprazolam is ordinarily widely distributed throughout the body tissues and across the blood-brain barrier. In this study, there are reasonably uniform concentrations of the drug between the brain, heart, kidney and liver. As seen by the data in this study, alprazolam in the brain is at a higher concentration than that in the plasma; the brain concentration reached 2750 ng/g at a dose of 10 mg/g of alprazolam. There was a reasonable relationship between the quantity of drug administered and the plasma concentration; 4.8 mg/kg gave a plasma concentration of 650 ng/mL, 7.8 mg/kg gave a plasma concentration of 950 ng/mL and 10 mg/kg gave a plasma concentration of 1450 ng/mL. At the end of 24 hours postmortem, the plasma concentration decreased to about one-half its initial level. This is possibly due to the partitioning of the drug into the more fatty tissue. It is difficult to conclude anything from the heart and liver concentrations as they were variable in both directions over time.

Atenolol Postmortem Studies:

The standard curve graphs for atenolol in heart, kidney, brain, liver and plasma are shown in Figure 46. A time series plot after administration of three different doses of atenolol (30 mg/kg, 25 mg/kg, and 35 mg/kg) show drug levels in the plasma in Figure 47; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The overall trend is for an increase in drug concentration in the plasma, with the exception of the highest dose, where the increase up to 24 hours is followed by a decrease at 48 hours.

A time series plot after administration of three different doses of atenolol (30 mg/kg, 25 mg/kg and 35 mg/kg) show drug levels in the brain in Figure 48; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The results generally show an increase in drug concentration in the brain from near zero initially to about 1500 ng/g after 48 hours. It is interesting that these increases appear to be zero order.

A time series plot after administration of three different doses of atenolol (30 mg/kg, 25 mg/kg and 35 mg/kg) show drug levels in the heart in Figure 49; the upper graph is a point-to-

point graph and the lower figure is a second-order regression plot. Overall, with the lower two doses, the atenolol concentrations do not change much over 48 hours. However, the 35 mg/kg dose increases over 24 hours and then falls back to near the original concentration after 48 hours.

A time series plot after administration of three different doses of atenolol (30 mg/kg, 25 mg/kg and 35 mg/kg) show drug levels in the kidney in Figure 50; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. In the kidney, there is not much change over the 48 hours with atenolol. It is interesting to note that there does not really appear to be a direct dose: drug level relationship in the kidney.

A time series plot after administration of three different doses of atenolol (30 mg/kg, 25 mg/kg and 35 mg/kg) show drug levels in the liver in Figure 51; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The liver data shows an overall slight increase, but not outstanding. The highest dose responds similar to the heart level in an increase in drug concentration in the liver up to 24 hours followed by a decrease back to near-initial levels after 48 hours.

The composite plasma data for the three separate dosing studies are shown in Figure 52. The overall change appears to be a slight increase over time. The levels at the 24 hours time period are spread out quite a bit for the 30 mg/kg and 25 mg/kg doses. For the 35 mg/kg dose, there appears to be an increase up to 24 hours followed by a decrease after 48 hours. The atenolol levels are mostly in the range of about 1,000-10,000 ng/g.

The composite brain data for the three separate dosing studies are shown in Figure 53. The overall trend in all three cases is a slight increase over the entire 48 hour time period. The 30 mg/kg dose does appear to be somewhat level, however, between 24 and 48 hours. The levels in the brain initially were very low, less than about 200 ng/g, increasing to about 1000 -2000 ng/g over 48 hours.

The heart data in Figure 54 shows some variability but not much in the way of consistent change. Initial concentrations for the 30 mg/kg dose were in the range of about 3000 ng/g increasing only to about 4000 ng/g after 48 hours. The 25 mg/kg dose started at about 3000 ng/g, increasing to about 6000 ng/g and the highest dose, 35 mg/kg, started at about 5000 ng/kg, increasing only to about 6000 ng/g.

The composite kidney data in Figure 55 showed very little change at the two higher doses over the 48 hour postmortem time period. The 30 mg/kg dose showed an increase at 24 hours, followed by a decrease after 48 hours. The low dose, 25 mg/kg, produced a level profile up to 48 hours, where a couple of points increased and a couple remained about the same. The high dose, 35 mg/kg showed little change, remaining in the range of about 15,000 ng/g.

The composite liver data in Figure 56 showed some variability between samples at specific time periods. Initial levels ranged from about 5,000 to about 6,000 ng/g. The 35 mg/kg dose

does show an increase after 24 hours followed by a decrease to near initial levels after 48 hours.

The middle dose (30 mg/kg) studies are plotted in a composite tissue plot, point-to-point, in Figure 57 and as a second-order regression plot in Figure 58. This plot shows relatively constant values for the tissues at a 30 mg/kg dose, with some variability seen for the kidney. Overall, there was not much change over the 48 hours period at the 30 mg/kg dose.

The low dose (25 mg/kg) studies are plotted in a composite tissue plot, point-to-point, in Figure 59 and as a second-order regression plot in Figure 60. It appears that the atenolol concentration remains fairly constant, with a slight increase over time in all tissues, and a larger increase for the kidney.

The high dose studies (35 mg/kg) are plotted in a composite tissue plot, point-to-point, in Figure 61 and as a second-order regression plot in Figure 62. These plots show a relatively slow, constant increase for atenolol in the brain, a larger increase up to 24 hours of atenolol in the liver, plasma and heart, and some fluctuation in the kidney, an increase, decrease, increase and another decrease.

There was a nice relationship between the dose administered and the plasma concentrations obtained; 25 mg/kg yielded plasma concentrations of about 2,000 ng/g, 30 mg/kg yielded plasma concentrations of about 2,500 ng/g and 35 mg/kg gave plasma concentrations of about 3,500 ng/g.

All three dosages of atenolol had similar distributions with the highest in the kidney and the lowest in the brain. The heart and liver show little change over 48 hours but the plasma increase was about two-fold. The very high level in the kidney is possibly due to the low o/w partition coefficient at physiological pH of the drug.

Atenolol is ordinarily well distributed in most tissues, except the brain. In this project, an initial concentration was near zero with an increase after 6 hours. This may be due to a loss of integrity of the blood-brain barrier, resulting in an influx of atenolol into the brain tissues.

Terfenadine Postmortem Studies:

The standard curve graphs for terfenadine in heart, kidney, brain, liver and plasma are shown in Figure 63.

A time series plot after administration of three different doses of terfenadine (6 mg/kg, 8 mg/kg and 10 mg/kg) show drug levels in the plasma in Figure 64; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. There appears to be an increase in plasma levels of terfenadine during the first few hours postmortem followed by a "leveling out" with the smaller dose and some increases up to 24 hours followed by decreases out

to 48 hours for the middle and high doses. (Note: In this case at 48 hours, there was interference at the internal standard retention time. Therefore, the internal standard concentrations used was an average of the previous plasma samples internal standard levels.)

A time series plot after administration of three different doses of terfenadine (6 mg/kg, 8 mg/kg and 10 mg/kg) show drug levels in the brain in Figure 65; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. Not much change occurs with terfenadine in the brain over the 48 hours postmortem.

A time series plot after administration of three different doses of terfenadine (6 mg/kg, 8 mg/kg and 10 mg/kg) show drug levels in the heart in Figure 66; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. There is a drop in the terfenadine concentrations in the heart between 1 and 6 hours postmortem, followed by a "resumption" of the initial levels that appear to remain constant out to 48 hours.

A time series plot after administration of three different doses of terfenadine (6 mg/kg, 8 mg/kg and 10 mg/kg) show drug levels in the kidney in Figure 67; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. More variability is seen in the kidney as a clear dose:drug level response is observed. The changes in terfenadine concentration appear to be limited, with an initial drop at the 6 hours followed by a leveling off for the 6 and 8 mg dose and an increase out to 24 hours followed by a slight decrease at 48 hours for the 10 mg/kg dose.

A time series plot after administration of three different doses of terfenadine (6 mg/kg, 8 mg/kg and 10 mg/kg) show drug levels in the liver in Figure 68; the upper graph is a point-to-point graph and the lower figure is a second-order regression plot. The dose:drug level response is again illustrated in the liver with overall relative constant concentrations of terfenadine throughout the 48 hour postmortem time period.

The composite plasma data for the three separate dosing studies for terfenadine are shown in Figure 69. The 6 mg/kg dose shows a slight overall increase in plasma concentration over the 48 hours but the data has some scatter. The initial concentrations of about 150 ng/mL increase to about 250 ng/mL over the 48 hours. For the 8 mg/kg dose, the initial 150 mg/mL terfenadine concentration increased after 1 hour to about 300 ng/mL and then remained relatively constant. The 10 mg/kg dose started at about 250 ng/mL and increased about two-fold before dropping to initial levels after 48 hours.

The composite brain data for the three separate dosing studies for terfenadine are shown in Figure 70. At the earlier time intervals, there is considerable scatter in the results. There is, however, a dose:drug level response ranging from about 1000 ng/g for the 6 mg/kg dose to about 1500 ng/g for the 8 mg/kg dose and about 2500 ng/g for the 10 mg/kg dose initially. Overall, it appears as if there is a very slight decrease in brain concentration of terfenadine over the 48 hour postmortem period.

The composite heart data for the three separate dosing studies for terfenadine are shown in Figure 71. Again, there is nice correlation at the initial time period with an increase in dose resulting in an increase in heart terfenadine concentration, ranging from about 3500 ng/g to about 8000 ng/g for the 6 and 10 mg/kg doses, respectively. The overall trend would appear to be a decrease in the concentration of terfenadine in the heart.

Figure 72 is the composite kidney data for the three separate dosing studies. The drug concentration dose relationship is present in the kidney, as well as in the previously mentioned tissues. It is interesting that the two lower doses produced a slight increase in drug concentration 1 hour postmortem and the high dose produced a decrease. Overall, throughout the 48 hour postmortem period, there appears to be a decrease in drug concentration in the kidney.

The composite liver data for the three separate dosing studies is shown in Figure 73. A good drug concentration:drug dose response was obtained with terfenadine levels ranging from about 1400 ng/g for the 6 mg/kg dose to about 3000 ng/g for the 10 mg/kg dose. There does not appear to be any remarkable alteration in drug concentration, however, over the 48 hour postmortem period in the liver.

The low dose studies are plotted in a composite tissue plot, point-to-point, in Figure 74 and as a second-order regression plot in Figure 75. These plots show very little change at the 6 mg/kg dose for the plasma, brain and liver. The kidney and heart demonstrated decreases in terfenadine 6 hours postmortem followed by slight increases where they remained constant from about 12 hours to 48 hours postmortem.

The medium dose (8 mg/kg) studies are plotted in a composite tissue plot, point-to-point, in Figure 76 and as a second-order regression plot in Figure 77. These results are similar to the low dose results with minimal changes occurring for the plasma, brain and liver and more fluctuation with the heart and kidney. With the exception of the increases after 1 hour, the overall trend for the kidney and heart was for a slight decrease in drug concentration.

The high dose studies for terfenadine are plotted as a composite plot, point-to-point in Figure 78 and as a second-order regression plot in Figure 79. Again, the plasma, brain and liver concentrations showed little change over the 48 hours but the heart and kidney were somewhat more variable with decreases after 1 and 6 hours, increases at 12 and 24 hours and a slight decrease at 48 hours postmortem.

Terfenadine concentration in the heart, liver and kidney show similar drug concentration trends with the kidney showing the highest concentration. In all tissues, there doesn't seem to be significant increase or decrease in terfenadine concentration over 48 hours. It is interesting that the lowest concentration of the drug was in the plasma. The brain concentration of the drug did not appreciably change with the increasing in dosing level. The level remained unchanged, even postmortem.

The most notable overall changes shown in this study include the following:

1. Postmortem diffusion into the blood was shown with atenolol.
2. Postmortem diffusion into the brain was shown with atenolol.
3. Postmortem diffusion from the blood was shown with alprazolam.
4. Postmortem diffusion from the brain was shown with alprazolam.
5. Greater variability occurred with alprazolam in the heart and liver from the aspect of increasing, decreasing and increasing concentrations over time. This was not as pronounced with atenolol or terfenadine.

It is now known that it is inappropriate to extrapolate drug dosages ingested from a single postmortem drug concentration as a large variance may exist between the blood and tissue concentration, especially during the postmortem interval of death to autopsy and site sampling. A number of factors involved in postmortem drug redistribution include postmortem interval, sampling sites, passive diffusion, volume of distribution and putrefaction (enzymatic degradation, microbial metabolism, pH changes). Not only may the drug itself be altered but the macromolecules and tissues to which the drug might be bound may be broken down, releasing the drug into the free state, resulting in a higher effective concentration in the tissue or fluid of interest. The migration of the free drug is thendependent upon its pKa, partition coefficient and the pH of the surrounding fluids. Much more detailed work needs to be done in this very important area.

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Table 1: Overal plan of the three phases for a multiyear project.

	YEAR				
	1	2	3	4	5
Phase I: Analytical methods development/ validation and antemortem and postmortem drug distribution in animal model.	Atenolol Triazolam Terfenadine	Metoprolol Diphenhydamine Alprazolam	Nadolol Triprolidine Lorazepam		
Phase II: Performance testing and correlation with antemortem levels.		Atenolol Triazolam Terfenadine	Metoprolol Diphenhydramine Alprazolam	Nadolol Triprolidine Lorazepam	
Phase III: High altitude effects on the pharmacokinetics of drugs.			Atenolol Triazolam Terfenadine	Metoprolol Diphenhydramine Alprazolam	Nadolol Triprolidine Lorazepam

Table 2: **Summary table of extraction procedures for the Hamilton Microlab 2200.**

	Alprazolam	Atenolol	Terfenadine
Precondition	2 mL MeOH 3 mL Water	2 mL MeOH 3 mL Water (pH 11)	2 mL MeOH 3 mL 0.1 M TEAA (pH 5)
Sample	0.5 mL of 2000 ng/mL	0.5 mL of 1000 ng/mL	0.5 mL of 4000 ng/mL
Rinse	1 mL of 0.1 M Ammonium Phosphate (pH 6.5)	2 mL Water	1 mL Water 1 mL Water:MeOH (7:3)
Elute	1 mL MeOH	1 mL Water (pH 3.5):MeOH (1:1)	0.5 mL MeOH 0.5 mL MeOH:TEAA (1:1), (pH 10)

Table 2 continued:

TREATMENT OF TISSUE:

Alprazolam:

200 µL water.
80 µL of 50,000 ng/mL stock (4000 ng/mL)
800 µL of 1 M TCA.

Vortex and centrifuge for 5 minutes at 5,000 rpm.
Pour the supernatant into a new tube.
Remove approximately 1 mL.
Load 0.5 mL (1000 ng) onto the column.

Atenolol:

200 µL liver homogenate.
10 µL of 100 µg/mL stock (1000 ng/mL)
800 µL of 1 M TCA.

Vortex and centrifuge for 5 minutes at 5000 rpm.
Pour supernatant to a new tube.
Obtain approximately 1 mL.
Load 0.5 mL (500 ng) onto the column.

Terfenadine:

200 µL liver homogenate
80 µL of 50,000 ng/mL stock (4000 ng/mL)
800 µL ACN

Vortex and centrifuge for 5 minutes at 5000 rpm.
Pour supernatant to a new tube.
Remove approximately 1 mL.
Load 0.5 mL (2000 ng) onto column.

Table 3 **HPLC analysis summary.**

	Alprazolam	Atenolol	Terfenadine
Mobile Phase	43.5% 0.05 M Ammonium Phosphate, pH 6.5, 43.5% MeOH, 13% ACN	13.29% ACN, 86.65% Water, 0.05% o-phosphoric acid, adjust to pH 3.5	6.25% 0.1 M TEAA, pH 5, 33% ACN, 60.75% MeOH
Wavelength	Ex: 220 Em: 280	Ex: 220 Em: 300	240 nm
Flow Rate	1 mL/minute	1 mL/minute	1 mL/minute
Column	C_8	CN	C_{18}

Table 4 **Intraday and interday validation studies for alprazolam, atenolol and terfenadine.**

Alprazolam	**Mean**	**Std. Dev.**	**%**
Day 1	1.11	0.051	4.64
Day 2	1.07	0.035	3.33
Day 3	1.09	0.045	4.13
Atenolol			
Day 1	0.23	0.004	1.74
Day 2	0.19	0.011	5.89
Day 3	0.19	0.008	4.04
Terfenadine			
Day 1	3.13	0.069	2.20
Day 2	2.97	0.064	2.15
Day 3	3.34	0.129	3.85

Table 5 **Alprazolam solid-phase extraction method using the Hamilton Microlab 2200.**

STEP 1: PRECONDITION WITH METHANOL

```
1     COMMENT************************************************************
2     COMMENT    --WATER IS PRIMED THRU SYRINGE 1&2.
3     COMMENT    --METHANOL IS PRIMED THRU SYRINGE 3.
5     COMMENT    --PRECONDITION BUFFER THRU SYRINGE 5.
6     COMMENT    --WASHING BUFFER THRU SYRINGE 6.
7     COMMENT    --ELUTION BUFFER THRU SYRINGE 4.
8     COMMENT    **TURN AIR PRESSURE TO 3.5 PSI**
10    COMMENT    **ENTER THE NUMBER OF SAMPLES**
15    ALARMC 2.
20    INPUT N.
30    MOVE TO STATION.
40    COMMENT    **INITIALIZING MVPS*********************************
50    ACTUATE DEV eXR.
70    WAIT 7.
120   COMMENT    **PICKING UP THE SPE HEAD FROM THE RACK**********
130   ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140   COMMENT    **PUSHING 2 mLS OF METHANOL THRU COLUMNS*********
150   FOR I=1 TO N.
155   FOR J=1 TO 2.
160   ASPIRATE 1000 UL OF DILUENT USING SYRINGE 3 AT SPEED 5.
170   DISPENSE 1000 UL TO S:SPE1(I) USING SYRINGE 3 AT SPEED 3.
180   MOVE TO S:SPE1(I) AT ZMAX.
190   COMMENT    **PUSH SOLUTION THRU COLUMN**********************
200   ACTUATE DEV eLPO2R.
210   WAIT 11.
220   ACTUATE DEV eLP11R.
230   WAIT 2.
235   NEXT J.
300   NEXT I.
350   END.
```

Table 5 Continued.

STEP 2: PRECONDITION WITH BUFFER

```
70      WASH 500 USING SYRINGE 5.
80      COMMENT     **PUSHING 3 mLS OF BUFFER #1 THROUGH COLUMNS***
90      FOR I=1 TO N.
95      FOR J=1 TO 3.
100     ASPIRATE 1000 UL OF DILUENT USING SYRINGE 5 AT SPEED 5.
110     DISPENSE 1000 UL OF S:SPE1(I) USING SYRINGE 5 AT SPEED 7.
120     MOVE TO S:SPE1(I) AT ZMAX.
130     COMMENT     ** PUSH SOLUTIONS THRU******************************
140     ACTUATE DEVeLPO2R.
150     WAIT 14.
160     ACTUATE DEVeLP11R.
170     WAIT 2.
175     NEXT J.
280     NEXT I.
285     COMMENT **PUTTING THE SPE HEAD BACK INTO RACK**************
390     ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
395     END.
```

Table 5 Continued.

STEP 3: LOAD SAMPLE

```
30      COMMENT **PICKING UP SAMPLE W/AIR GAP AND OVERFILL******
40      FOR I=1 TO N.
50      ASPIRATE 20 OF AIR USING SYRINGE 1.
60      ASPIRATE 520 UL OF SAMPLE(I) USING SYRINGE 1 AT SPEED 7.
70      COMMENT **DISPENSING SAMPLE TO COLUMN******************
80      DISPENSE 500 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 7.
90      WASH 1000 USING SYRINGE 1.
100     NEXT I.
130     COMMENT **PICKING UP THE SPE HEAD**************************
135     ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140     COMMENT **PUSHING THE SAMPLE THRU THE COLUMN TO WASTE**
145     FOR I=1 TO N.
150     MOVE TO S:SPE1(I) AT ZMAX.
155     ACTUATE DEVeLPO2R.
160     WAIT 13.
165     ACTUATE DEVeLP11R.
170     WAIT 2.
180     NEXT I.
210     END.
```

Table 5 Continued.

STEP 4: DRYING THE COLUMN

```
5       COMMENT*****************************************************
10      ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20      COMMENT **TURN AIR PRESSURE TO 5 PSI.
40      WAIT 30.
130     COMMENT ******DRYING THE COLUMN*************************
160     FOR I = 1 TO N.
170     MOVE TO S:SPE1(I) AT ZMAX.
214     ACTUATE DEVeLPO2R.
215     WAIT 20.
216     ACTUATE DEVeLP11R.
217     WAIT 2.
220     NEXT I.
230     COMMENT **PUTTING THE HEAD BACK INTO THE RACK*********
240     ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270     END.
```

Table 5 Continued.

STEP 5: RINSING THE COLUMN WITH BUFFER

```
5       COMMENT ***PICKING UP THE SPE HEAD************************
10      ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20      COMMENT ***RINSING COLUMN WITH 1 mL OF WATER TO WASTE**
25      FOR I = 1 TO N.
30      ASPIRATE 1000 UL OF DILUENT USING SYRINGE 1 AT SPEED 7.
40      DISPENSE 1000 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 4.
50      MOVE TO S:SPE1(I) AT ZMAX.
60      ACTUATE DEVeLP02R.
70      WAIT 45.
80      ACTUATE DEVeLP11R.
90      WAIT 2.
100     NEXT I.
150     COMMENT **RINSING WITH 1 mL OF (30:70) MEOH:WATER**********
160     FOR I = 1 TO N.
205     MULTIASPIRATE 300,700 UL OF DILUENT USING SYRINGE 3,1 AT SPEED 7,5.
210     MULTIDISPENSE 300,700 TO S:SPE1(I) USING SYRINGE 3,1 AT SPEED 5,2.
220     MOVE TO S:SPE1(I) AT ZMAX.
230     ACTUATE DEVeLPO2R.
240     WAIT 45.
250     ACTUATE DEVeLP11R.
260     WAIT 3.
262     NEXT I.
270     COMMENT **PUTTING THE HEAD BACK TO RACK*****************
280     ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
350     END.
```

Table 5 Continued

STEP 6: DRYING THE COLUMN

```
5       COMMENT*****************************************************
10      ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20      COMMENT **TURN AIR PRESSURE TO 5 PSI.
40      WAIT 30.
130     COMMENT ******DRYING THE COLUMN**************************
160     FOR I = 1 TO N.
170     MOVE TO S:SPE1(I) AT ZMAX.
214     ACTUATE DEVeLPO2R.
215     WAIT 20.
216     ACTUATE DEVeLP11R.
217     WAIT 2.
220     NEXT I.
230     COMMENT **PUTTING THE HEAD BACK INTO THE RACK***********
240     ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270     END.
```

Table 5 **Continued.**

STEP 7: ELUTING THE SAMPLE

```
30    COMMENT***MOVING THE COLUMN CARRIER OVER COLLECTION RACK**
40    ACTUATE ARM POSF1, POSG1, POSH1, POSI1.
50    COMMENT ***PICKING THE HEAD BACK UP*****************************
60    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
70    COMMENT ***DECREASE AIR PRESSURE BACK TO 3 PSI*****************
80    ALARMC 5.
90    WAIT 15.
130   COMMENT ***ELUTING THE SAMPLE---SYRINGE 4**********************
140   FOR I = 1 TO N.
145   FOR J = 1 TO 2.
150   ASPIRATE 500 OF DILUENT USING SYRINGE 4 AT SPEED 5.
160   DISPENSE 500 TO S:SPE2 (I) USING SYRINGE 4 AT SPEED 7.
170   MOVE TO S:SPE2 (I) AT ZMAX.
180   ACTUATE DEVeLPO2R.
190   WAIT 20.
195   ACTUATE DEVeLP11R.
200   WAIT 2.
210   NEXT J.
290   NEXT I.
305   COMMENT ****TURN AIR UP TO 5 PSI TO DRY COLUMN***************
309   ALARMC 5.
310   WAIT 10.
315   FOR I = 1 TO N.
318   MOVE TO S:SPE2(I) AT ZMAX.
320   ACTUATE DEVeLPO2R.
330   WAIT 10.
340   ACTUATE DEVeLP11R.
350   WAIT 2.
360   NEXT I.
370   COMMENT ***PUTTING THE HEAD BACK INTO THE RACK*************
380   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
390   COMMENT **MOVING CARTRIDGE CARRIER BACK TO START POINT**
400   ACTUATE ARM POSJ1, POSK1, POSL1, POSM1.
440   END.
```

Table 6 **Atenolol solid-phase extraction method using the Hamilton Microlab 2200.**

STEP 1: PRECONDITION WITH METHANOL

```
1     COMMENT************************************************************
2     COMMENT    --WATER IS PRIMED THRU SYRINGE 1&2.
3     COMMENT    --METHANOL IS PRIMED THRU SYRINGE 3.
5     COMMENT    --PRECONDITION BUFFER THRU SYRINGE 5.
6     COMMENT    --WASHING BUFFER THRU SYRINGE 6.
7     COMMENT    --ELUTION BUFFER THRU SYRINGE 4.
8     COMMENT    **TURN AIR PRESSURE TO 3.5 PSI**
10    COMMENT    **ENTER THE NUMBER OF SAMPLES**
15    ALARMC 2.
20    INPUT N.
30    MOVE TO STATION.
40    COMMENT    **INITIALIZING MVPS**********************************
50    ACTUATE DEV eXR.
70    WAIT 7.
120   COMMENT    **PICKING UP THE SPE HEAD FROM THE RACK**********
130   ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140   COMMENT    **PUSHING 2 mLS OF METHANOL THRU COLUMNS*********
150   FOR I=1 TO N.
155   FOR J=1 TO 2.
160   ASPIRATE 1000 UL OF DILUENT USING SYRINGE 3 AT SPEED 5.
170   DISPENSE 1000 UL TO S:SPE1(I) USING SYRINGE 3 AT SPEED 3.
180   MOVE TO S:SPE1(I) AT ZMAX.
190   COMMENT    **PUSH SOLUTION THRU COLUMN**********************
200   ACTUATE DEV eLPO2R.
210   WAIT 11.
220   ACTUATE DEV eLP11R.
230   WAIT 2.
235   NEXT J.
300   NEXT I.
350   END.
```

Table 6 Continued.

STEP 2: PRECONDITION WITH BUFFER

```
70    WASH 500 USING SYRINGE 5.
80    COMMENT    **PUSHING 3 mLS OF BUFFER #1 THROUGH COLUMNS***
90    FOR I=1 TO N.
95    FOR J=1 TO 3.
100   ASPIRATE 1000 UL OF DILUENT USING SYRINGE 5 AT SPEED 5.
110   DISPENSE 1000 UL OF S:SPE1(I) USING SYRINGE 5 AT SPEED 7.
120   MOVE TO S:SPE1(I) AT ZMAX.
130   COMMENT    ** PUSH SOLUTIONS THRU******************************
140   ACTUATE DEVeLPO2R.
150   WAIT 14.
160   ACTUATE DEVeLP11R.
170   WAIT 2.
175   NEXT J.
280   NEXT I.
285   COMMENT **PUTTING THE SPE HEAD BACK INTO RACK**********
390   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
395   END.
```

Table 6 Continued.

STEP 3: LOAD SAMPLE

```
30    COMMENT **PICKING UP SAMPLE W/AIR GAP AND OVERFILL******
40    FOR I=1 TO N.
50    ASPIRATE 20 OF AIR USING SYRINGE 1.
60    ASPIRATE 520 UL OF SAMPLE(I) USING SYRINGE 1 AT SPEED 7.
70    COMMENT **DISPENSING SAMPLE TO COLUMN******************
80    DISPENSE 500 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 7.
90    WASH 1000 USING SYRINGE 1.
100   NEXT I.
130   COMMENT **PICKING UP THE SPE HEAD*************************
135   ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140   COMMENT **PUSHING THE SAMPLE THRU THE COLUMN TO WASTE**
145   FOR I=1 TO N.
150   MOVE TO S:SPE1(I) AT ZMAX.
155   ACTUATE DEVeLPO2R.
160   WAIT 13.
165   ACTUATE DEVeLP11R.
170   WAIT 2.
180   NEXT I.
210   END.
```

Table 6 Continued.

STEP 4: DRYING THE COLUMN

```
5     COMMENT*****************************************************
10    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20    COMMENT **TURN AIR PRESSURE TO 5 PSI.
40    WAIT 30.
130   COMMENT ******DRYING THE COLUMN**************************
160   FOR I = 1 TO N.
170   MOVE TO S:SPE1(I) AT ZMAX.
214   ACTUATE DEVeLPO2R.
215   WAIT 20.
216   ACTUATE DEVeLP11R.
217   WAIT 2.
220   NEXT I.
230   COMMENT **PUTTING THE HEAD BACK INTO THE RACK*********
240   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270   END.
```

Table 6 **Continued.**

STEP 5: RINSING THE COLUMN WITH BUFFER

```
5     COMMENT ***PICKING UP THE SPE HEAD*************************
10    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20    COMMENT ***RINSING COLUMN WITH 1 mL OF WATER TO WASTE**
25    FOR I = 1 TO N.
30    ASPIRATE 1000 UL OF DILUENT USING SYRINGE 1 AT SPEED 7.
40    DISPENSE 1000 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 4.
50    MOVE TO S:SPE1(I) AT ZMAX.
60    ACTUATE DEVeLP02R.
70    WAIT 45.
80    ACTUATE DEVeLP11R.
90    WAIT 2.
100   NEXT I.
150   COMMENT **RINSING WITH 1 mL OF (30:70) MEOH:WATER**********
160   FOR I = 1 TO N.
205   MULTIASPIRATE 300,700 UL OF DILUENT USING SYRINGE 3,1 AT SPEED 7,5.
210   MULTIDISPENSE 300,700 TO S:SPE1(I) USING SYRINGE 3,1 AT SPEED 5,2.
220   MOVE TO S:SPE1(I) AT ZMAX.
230   ACTUATE DEVeLPO2R.
240   WAIT 45.
250   ACTUATE DEVeLP11R.
260   WAIT 3.
262   NEXT I.
270   COMMENT **PUTTING THE HEAD BACK TO RACK*****************
280   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
350   END.
```

Table 6 **Continued**

STEP 6: DRYING THE COLUMN

```
5       COMMENT*****************************************************
10      ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20      COMMENT **TURN AIR PRESSURE TO 5 PSI.
40      WAIT 30.
130     COMMENT ******DRYING THE COLUMN**************************
160     FOR I = 1 TO N.
170     MOVE TO S:SPE1(I) AT ZMAX.
214     ACTUATE DEVeLPO2R.
215     WAIT 20.
216     ACTUATE DEVeLP11R.
217     WAIT 2.
220     NEXT I.
230     COMMENT **PUTTING THE HEAD BACK INTO THE RACK**********
240     ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270     END.
```

Table 6 **Continued.**

STEP 7: ELUTING THE SAMPLE

```
30    COMMENT***MOVING THE COLUMN CARRIER OVER COLLECTION RACK**
40    ACTUATE ARM POSF1, POSG1, POSH1, POSI1.
50    COMMENT ***PICKING THE HEAD BACK UP*****************************
60    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
70    COMMENT ***DECREASE AIR PRESSURE BACK TO 3 PSI*****************
80    ALARMC 5.
90    WAIT 15.
130   COMMENT ***ELUTING THE SAMPLE---SYRINGE 4**********************
140   FOR I = 1 TO N.
145   FOR J = 1 TO 2.
150   ASPIRATE 500 OF DILUENT USING SYRINGE 4 AT SPEED 5.
160   DISPENSE 500 TO S:SPE2 (I) USING SYRINGE 4 AT SPEED 7.
170   MOVE TO S:SPE2 (I) AT ZMAX.
180   ACTUATE DEVeLPO2R.
190   WAIT 20.
195   ACTUATE DEVeLP11R.
200   WAIT 2.
210   NEXT J.
290   NEXT I.
305   COMMENT ****TURN AIR UP TO 5 PSI TO DRY COLUMN***************
309   ALARMC 5.
310   WAIT 10.
315   FOR I = 1 TO N.
318   MOVE TO S:SPE2(I) AT ZMAX.
320   ACTUATE DEVeLPO2R.
330   WAIT 10.
340   ACTUATE DEVeLP11R.
350   WAIT 2.
360   NEXT I.
370   COMMENT ***PUTTING THE HEAD BACK INTO THE RACK*************
380   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
390   COMMENT **MOVING CARTRIDGE CARRIER BACK TO START POINT**
400   ACTUATE ARM POSJ1, POSK1, POSL1, POSM1.
440   END.
```

Table 7 **Terfenadine solid-phase extraction method using the Hamilton Microlab 2200.**

STEP 1: PRECONDITION WITH METHANOL

```
1     COMMENT************************************************************
2     COMMENT    --WATER IS PRIMED THRU SYRINGE 1&2.
3     COMMENT    --METHANOL IS PRIMED THRU SYRINGE 3.
5     COMMENT    --PRECONDITION BUFFER THRU SYRINGE 5.
6     COMMENT    --WASHING BUFFER THRU SYRINGE 6.
7     COMMENT    --ELUTION BUFFER THRU SYRINGE 4.
8     COMMENT    **TURN AIR PRESSURE TO 3.5 PSI**
10    COMMENT    **ENTER THE NUMBER OF SAMPLES**
15    ALARMC 2.
20    INPUT N.
30    MOVE TO STATION.
40    COMMENT    **INITIALIZING MVPS**********************************
50    ACTUATE DEV eXR.
70    WAIT 7.
120   COMMENT    **PICKING UP THE SPE HEAD FROM THE RACK**********
130   ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140   COMMENT    **PUSHING 2 mLS OF METHANOL THRU COLUMNS*********
150   FOR I=1 TO N.
155   FOR J=1 TO 2.
160   ASPIRATE 1000 UL OF DILUENT USING SYRINGE 3 AT SPEED 5.
170   DISPENSE 1000 UL TO S:SPE1(I) USING SYRINGE 3 AT SPEED 3.
180   MOVE TO S:SPE1(I) AT ZMAX.
190   COMMENT    **PUSH SOLUTION THRU COLUMN**********************
200   ACTUATE DEV eLPO2R.
210   WAIT 11.
220   ACTUATE DEV eLP11R.
230   WAIT 2.
235   NEXT J.
300   NEXT I.
350   END.
```

Table 7 Continued.

STEP 2: PRECONDITION WITH BUFFER

```
70    WASH 500 USING SYRINGE 5.
80    COMMENT    **PUSHING 3 mLS OF BUFFER #1 THROUGH COLUMNS***
90    FOR I=1 TO N.
95    FOR J=1 TO 3.
100   ASPIRATE 1000 UL OF DILUENT USING SYRINGE 5 AT SPEED 5.
110   DISPENSE 1000 UL OF S:SPE1(I) USING SYRINGE 5 AT SPEED 7.
120   MOVE TO S:SPE1(I) AT ZMAX.
130   COMMENT    ** PUSH SOLUTIONS THRU******************************
140   ACTUATE DEVeLPO2R.
150   WAIT 14.
160   ACTUATE DEVeLP11R.
170   WAIT 2.
175   NEXT J.
280   NEXT I.
285   COMMENT **PUTTING THE SPE HEAD BACK INTO RACK**************
390   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
395   END.
```

Table 7 Continued.

STEP 3: LOAD SAMPLE

```
30    COMMENT **PICKING UP SAMPLE W/AIR GAP AND OVERFILL******
40    FOR I=1 TO N.
50    ASPIRATE 20 OF AIR USING SYRINGE 1.
60    ASPIRATE 520 UL OF SAMPLE(I) USING SYRINGE 1 AT SPEED 7.
70    COMMENT **DISPENSING SAMPLE TO COLUMN******************
80    DISPENSE 500 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 7.
90    WASH 1000 USING SYRINGE 1.
100   NEXT I.
130   COMMENT **PICKING UP THE SPE HEAD**************************
135   ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
140   COMMENT **PUSHING THE SAMPLE THRU THE COLUMN TO WASTE**
145   FOR I=1 TO N.
150   MOVE TO S:SPE1(I) AT ZMAX.
155   ACTUATE DEVeLPO2R.
160   WAIT 13.
165   ACTUATE DEVeLP11R.
170   WAIT 2.
180   NEXT I.
210   END.
```

Table 7 **Continued.**

STEP 4: DRYING THE COLUMN

```
5     COMMENT*****************************************************
10    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20    COMMENT **TURN AIR PRESSURE TO 5 PSI.
40    WAIT 30.
130   COMMENT ******DRYING THE COLUMN*************************
160   FOR I = 1 TO N.
170   MOVE TO S:SPE1(I) AT ZMAX.
214   ACTUATE DEVeLPO2R.
215   WAIT 20.
216   ACTUATE DEVeLP11R.
217   WAIT 2.
220   NEXT I.
230   COMMENT **PUTTING THE HEAD BACK INTO THE RACK*********
240   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270   END.
```

Table 7 **Continued.**

STEP 5: RINSING THE COLUMN WITH BUFFER

```
5     COMMENT ***PICKING UP THE SPE HEAD**************************
10    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20    COMMENT ***RINSING COLUMN WITH 1 mL OF WATER TO WASTE**
25    FOR I = 1 TO N.
30    ASPIRATE 1000 UL OF DILUENT USING SYRINGE 1 AT SPEED 7.
40    DISPENSE 1000 TO S:SPE1(I) USING SYRINGE 1 AT SPEED 4.
50    MOVE TO S:SPE1(I) AT ZMAX.
60    ACTUATE DEVeLP02R.
70    WAIT 45.
80    ACTUATE DEVeLP11R.
90    WAIT 2.
100   NEXT I.
150   COMMENT **RINSING WITH 1 mL OF (30:70) MEOH:WATER**********
160   FOR I = 1 TO N.
205   MULTIASPIRATE 300,700 UL OF DILUENT USING SYRINGE 3,1 AT SPEED 7,5.
210   MULTIDISPENSE 300,700 TO S:SPE1(I) USING SYRINGE 3,1 AT SPEED 5,2.
220   MOVE TO S:SPE1(I) AT ZMAX.
230   ACTUATE DEVeLPO2R.
240   WAIT 45.
250   ACTUATE DEVeLP11R.
260   WAIT 3.
262   NEXT I.
270   COMMENT **PUTTING THE HEAD BACK TO RACK*****************
280   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
350   END.
```

Table 7 **Continued**

STEP 6: DRYING THE COLUMN

```
5     COMMENT*****************************************************
10    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
20    COMMENT **TURN AIR PRESSURE TO 5 PSI.
40    WAIT 30.
130   COMMENT ******DRYING THE COLUMN**************************
160   FOR I = 1 TO N.
170   MOVE TO S:SPE1(I) AT ZMAX.
214   ACTUATE DEVeLPO2R.
215   WAIT 20.
216   ACTUATE DEVeLP11R.
217   WAIT 2.
220   NEXT I.
230   COMMENT **PUTTING THE HEAD BACK INTO THE RACK**********
240   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
270   END.
```

Table 7 **Continued.**

STEP 7: ELUTING THE SAMPLE

```
30    COMMENT***MOVING THE COLUMN CARRIER OVER COLLECTION RACK**
40    ACTUATE ARM POSF1, POSG1, POSH1, POSI1.
50    COMMENT ***PICKING THE HEAD BACK UP*****************************
60    ACTUATE ARM POSA1, POSB1, POSC1, POSD1, POSE1.
70    COMMENT ***DECREASE AIR PRESSURE BACK TO 3 PSI*****************
80    ALARMC 5.
90    WAIT 15.
130   COMMENT ***ELUTING THE SAMPLE---SYRINGE 4**********************
140   FOR I = 1 TO N.
145   FOR J = 1 TO 2.
150   ASPIRATE 500 OF DILUENT USING SYRINGE 4 AT SPEED 5.
160   DISPENSE 500 TO S:SPE2 (I) USING SYRINGE 4 AT SPEED 7.
170   MOVE TO S:SPE2 (I) AT ZMAX.
180   ACTUATE DEVeLPO2R.
190   WAIT 20.
195   ACTUATE DEVeLP11R.
200   WAIT 2.
210   NEXT J.
290   NEXT I.
305   COMMENT ****TURN AIR UP TO 5 PSI TO DRY COLUMN***************
309   ALARMC 5.
310   WAIT 10.
315   FOR I = 1 TO N.
318   MOVE TO S:SPE2(I) AT ZMAX.
320   ACTUATE DEVeLPO2R.
330   WAIT 10.
340   ACTUATE DEVeLP11R.
350   WAIT 2.
360   NEXT I.
370   COMMENT ***PUTTING THE HEAD BACK INTO THE RACK*************
380   ACTUATE ARM POSE1, POSD1, POSC1, POSA1.
390   COMMENT **MOVING CARTRIDGE CARRIER BACK TO START POINT**
400   ACTUATE ARM POSJ1, POSK1, POSL1, POSM1.
440   END.
```

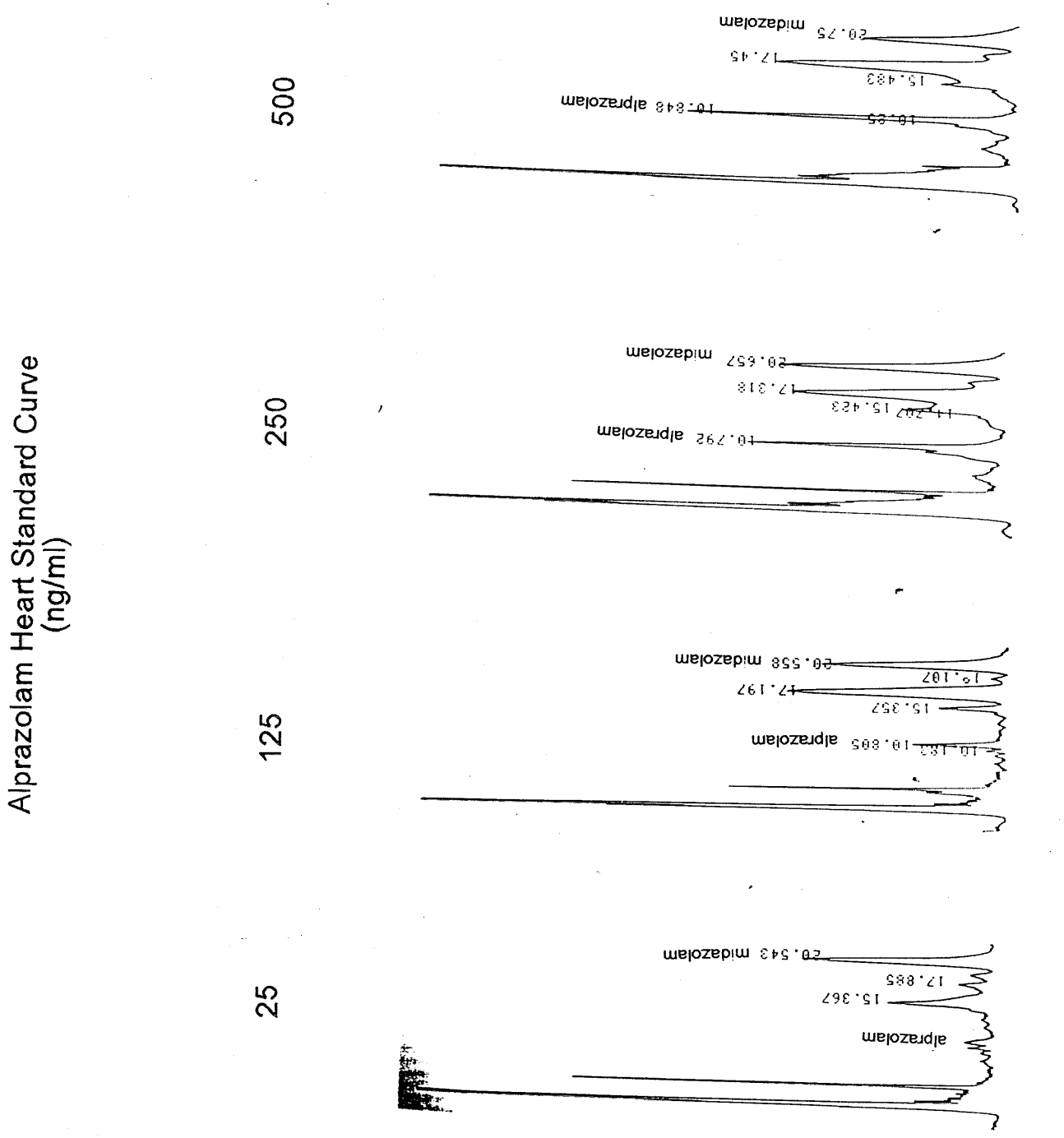


FIGURE 1. Standard Curve Chromatographs for Alprazolam in Heart: Concentrations of 25-500 ng/ml Using Midazolam as Internal Standard

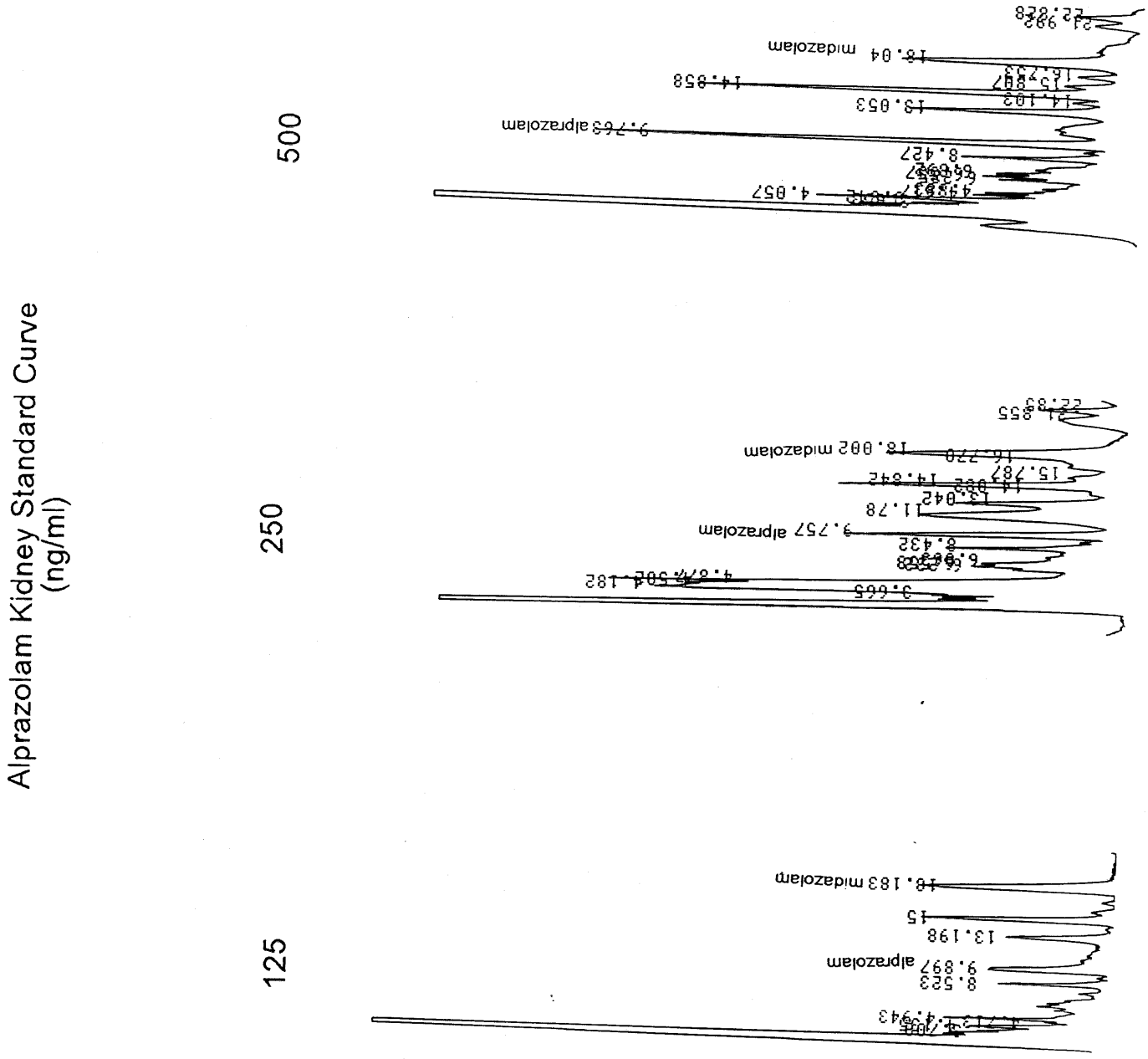


FIGURE 2. Standard Curve Chromatographs for Alprazolam in Kidney: Concentrations of 125-500 ng/ml Using Midazolam as Internal Standard

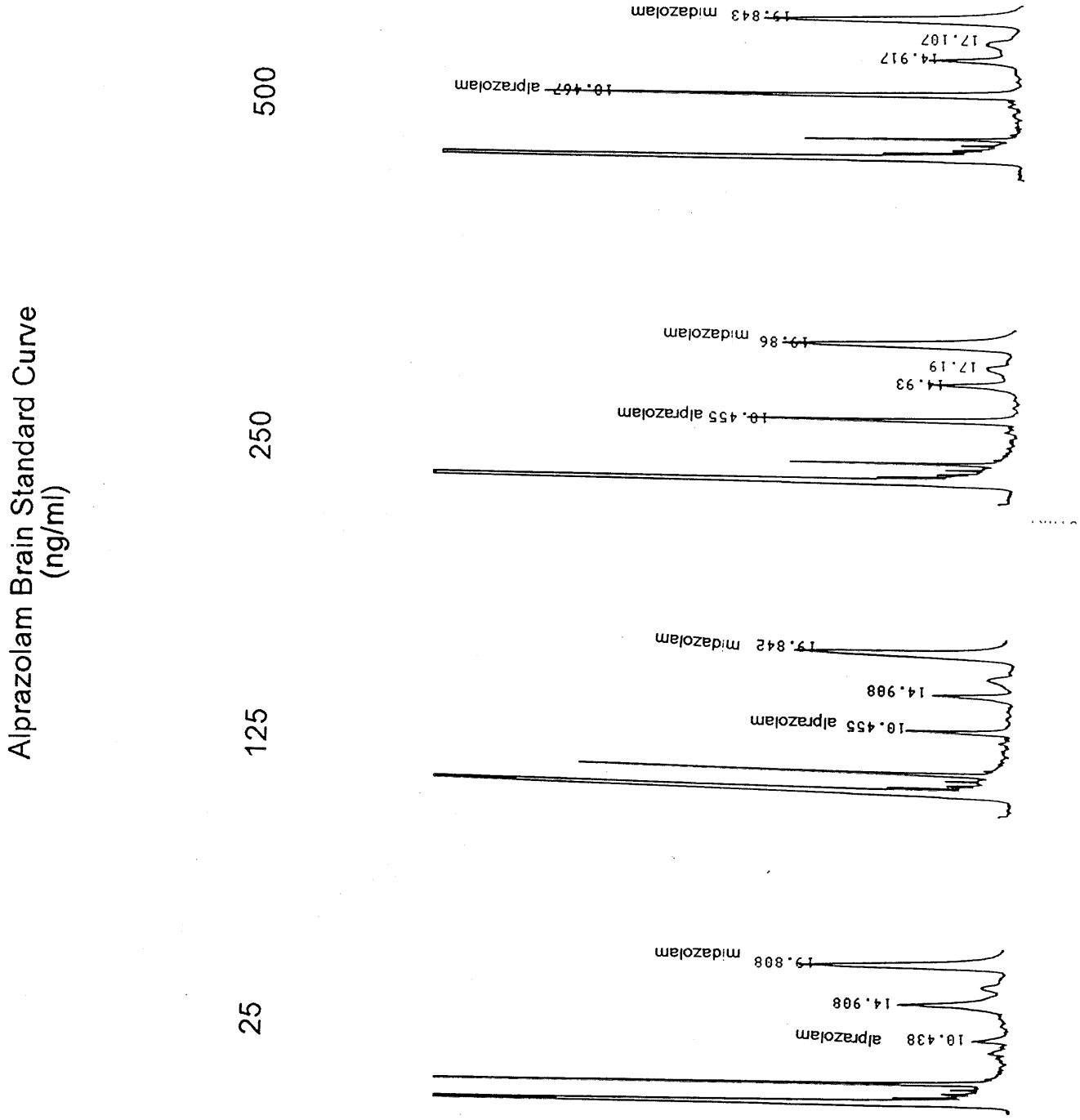


FIGURE 3. Standard Curve Chromatographs for Alprazolam in Brain: Concentrations of 25-500 ng/ml Using Midazolam as Internal Standard

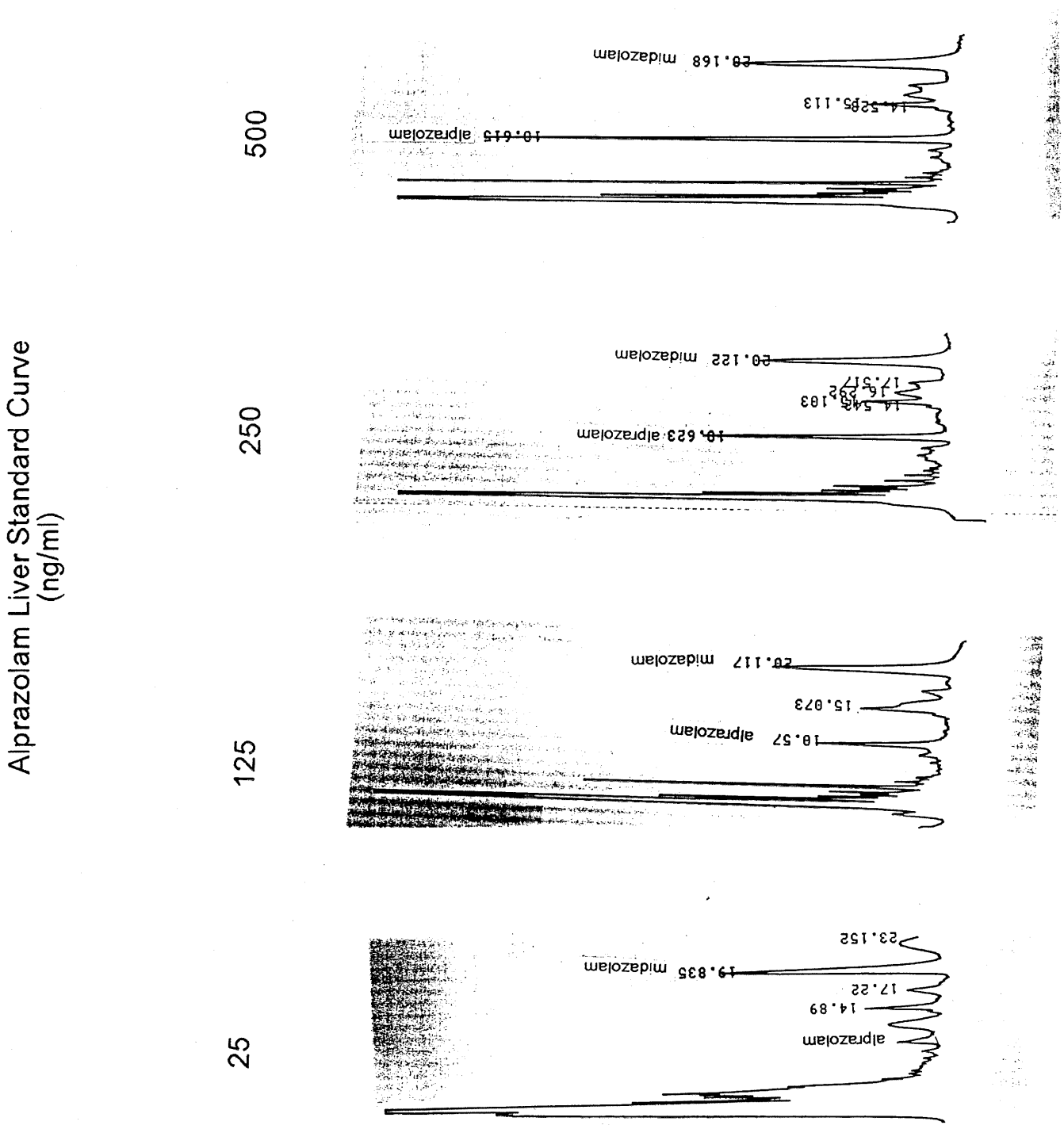


FIGURE 4. Standard Curve Chromatographs for Alprazolam in Liver: Concentrations of 25-500 ng/ml Using Midazolam as Internal Standard

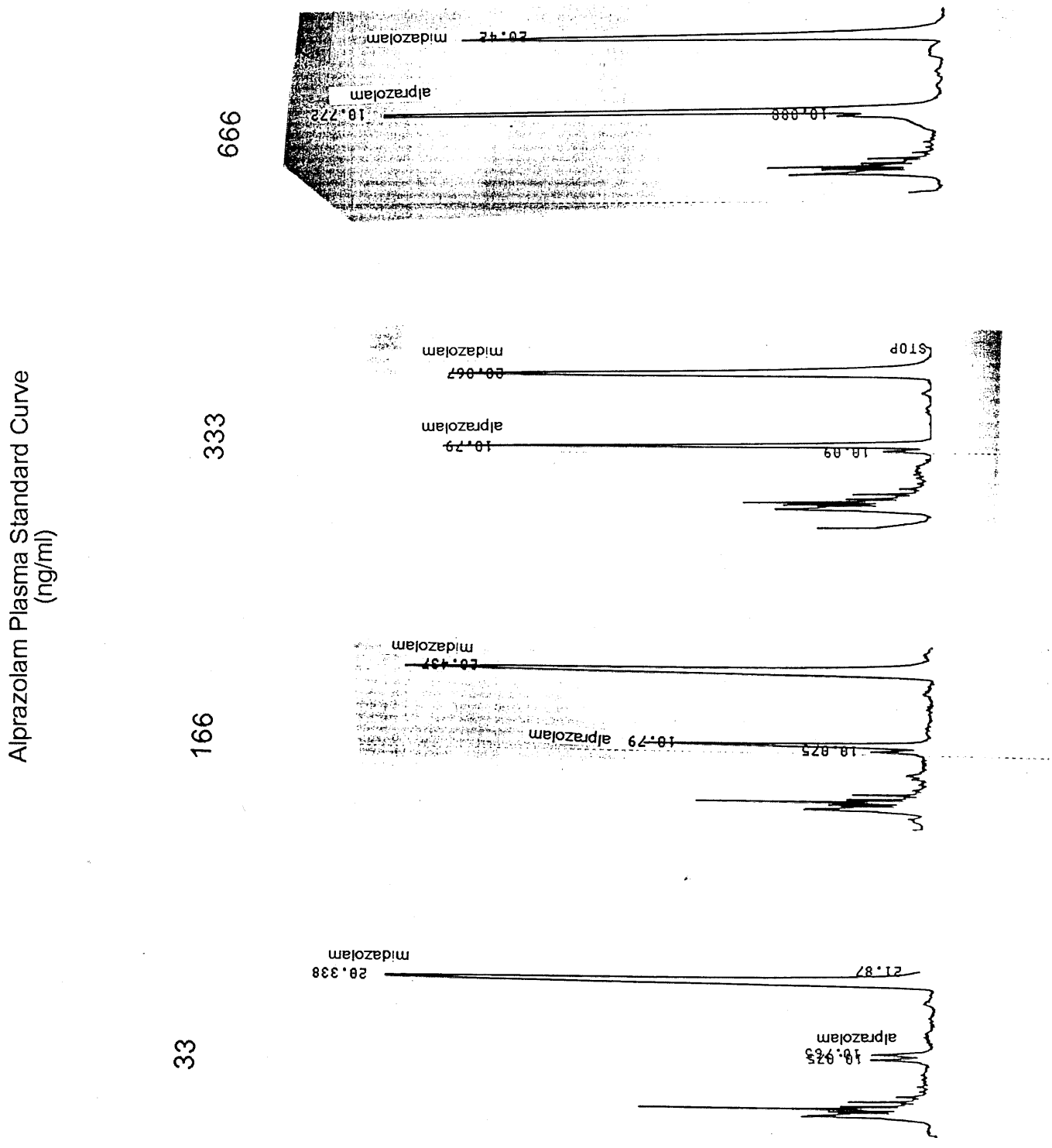


FIGURE 5. Standard Curve Chromatographs for Alprazolam in Plasma: Concentrations of 33-666 ng/ml Using Midazolam as Internal Standard

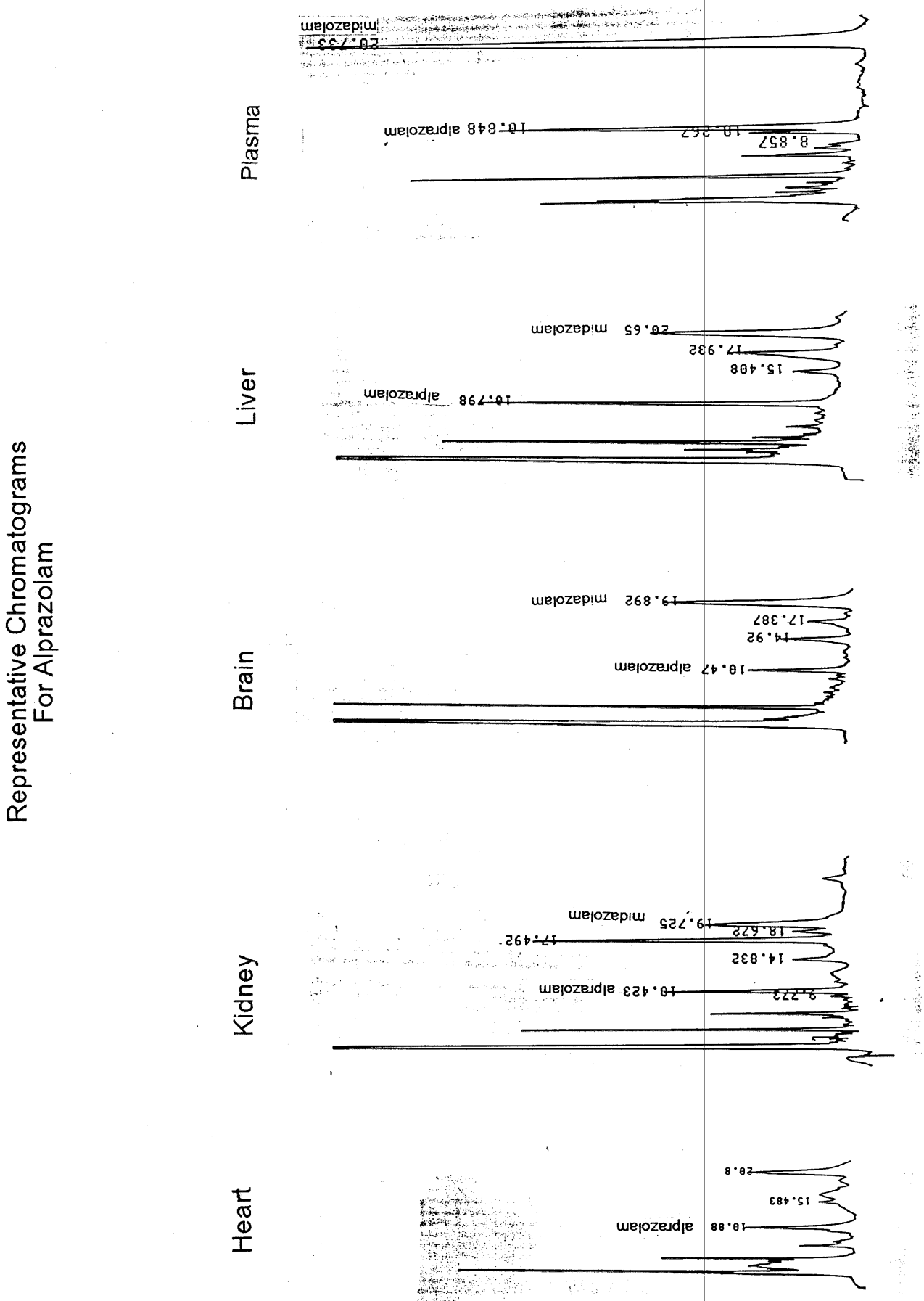


FIGURE 6. Representative Chromatograms for Alprazolam in Heart, Kidney, Brain, Liver and Plasma Using Midazolam as Internal Standard

FIGURE 7. Representative Chromatograms for Alprazolam Validation Using Midazolam as Internal Standard

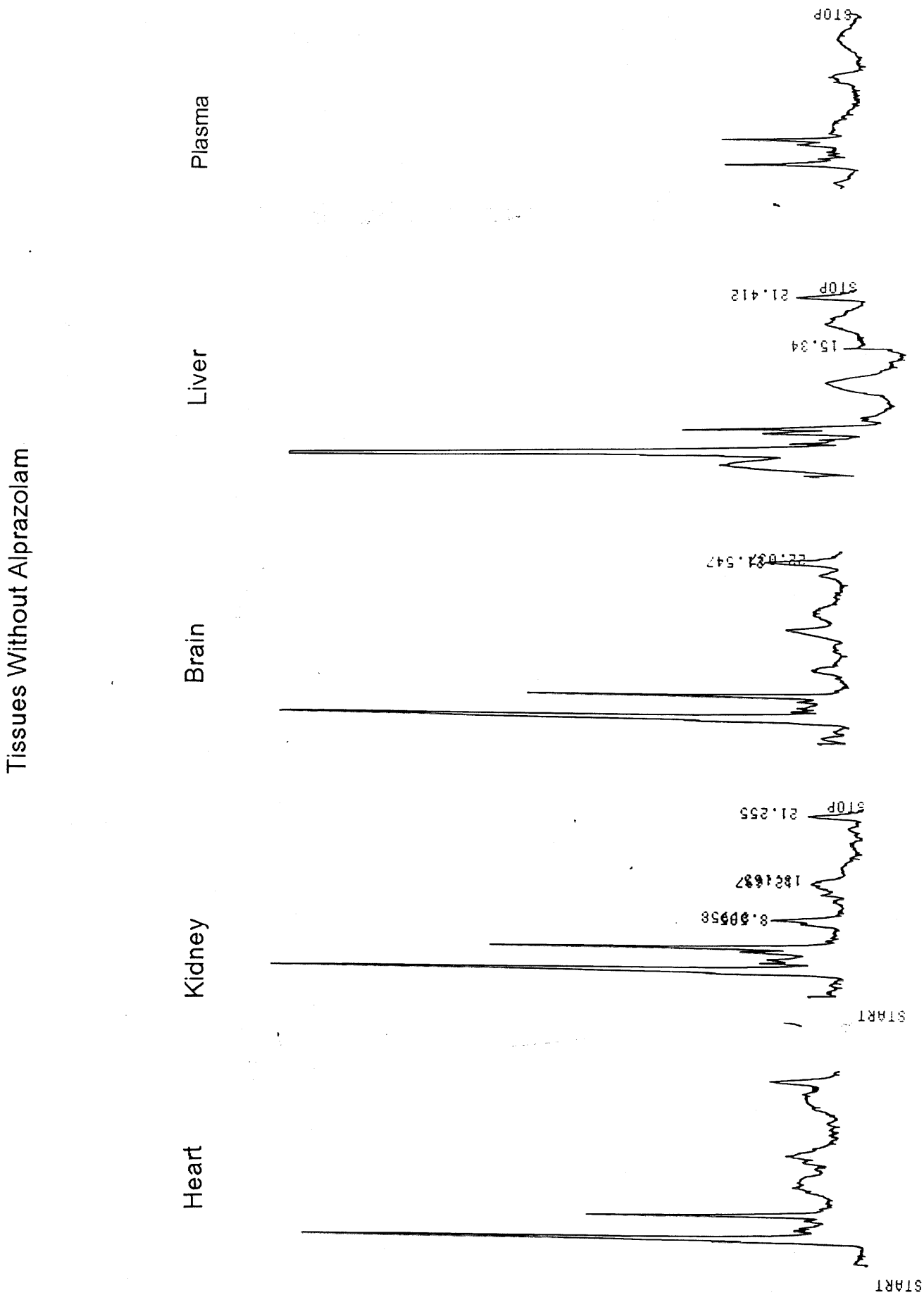


FIGURE 8. Representative Chromatograms for Héart, Kidney, Brain, Liver and Plasma Extraction Without Alprazolam

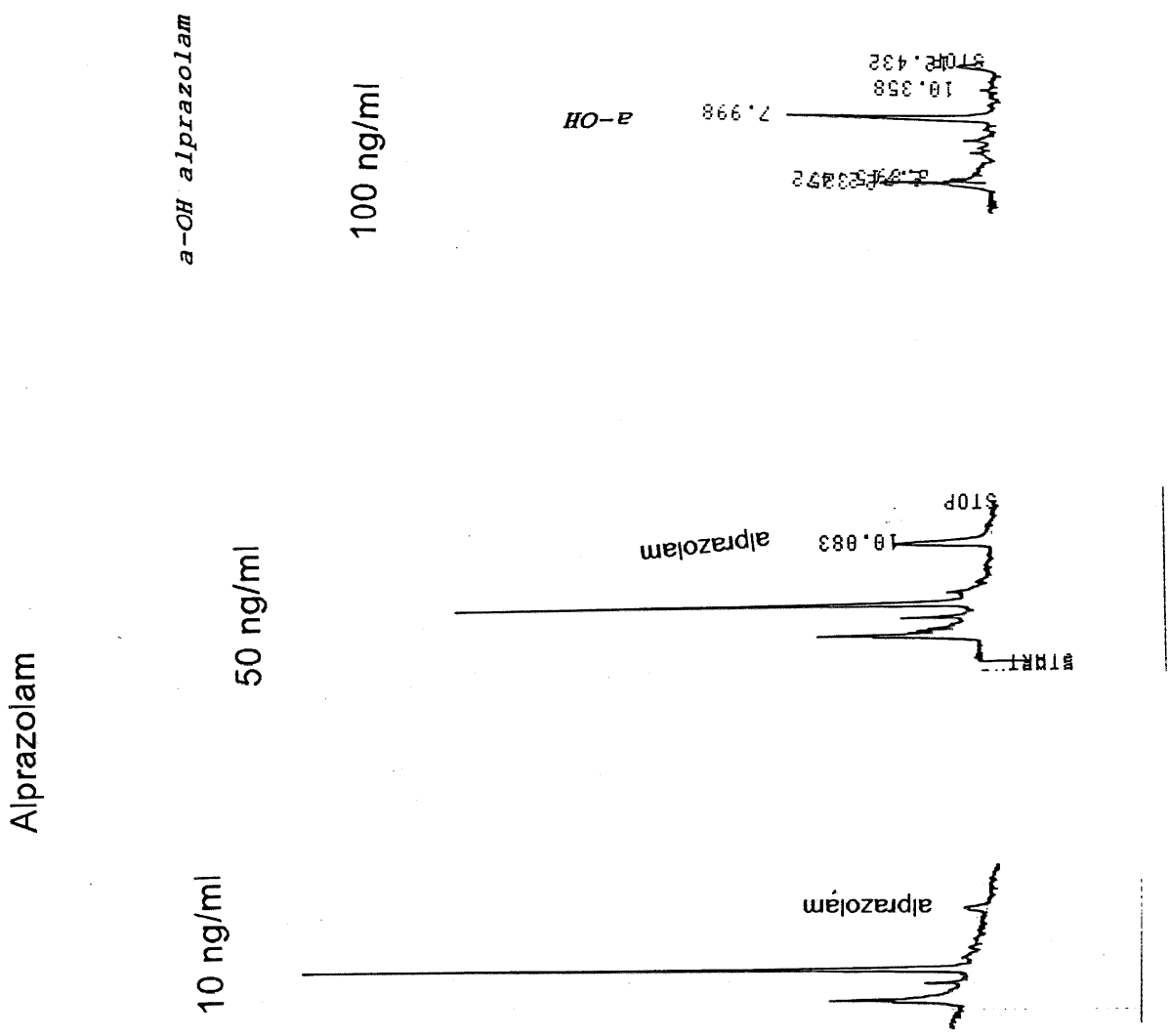


FIGURE 9. Representative Chromatograms of Alprazolam Standards at 10 and 50 ng/ml and its Metabolite, α-OH Alprazolam, Standard at 100 ng/ml

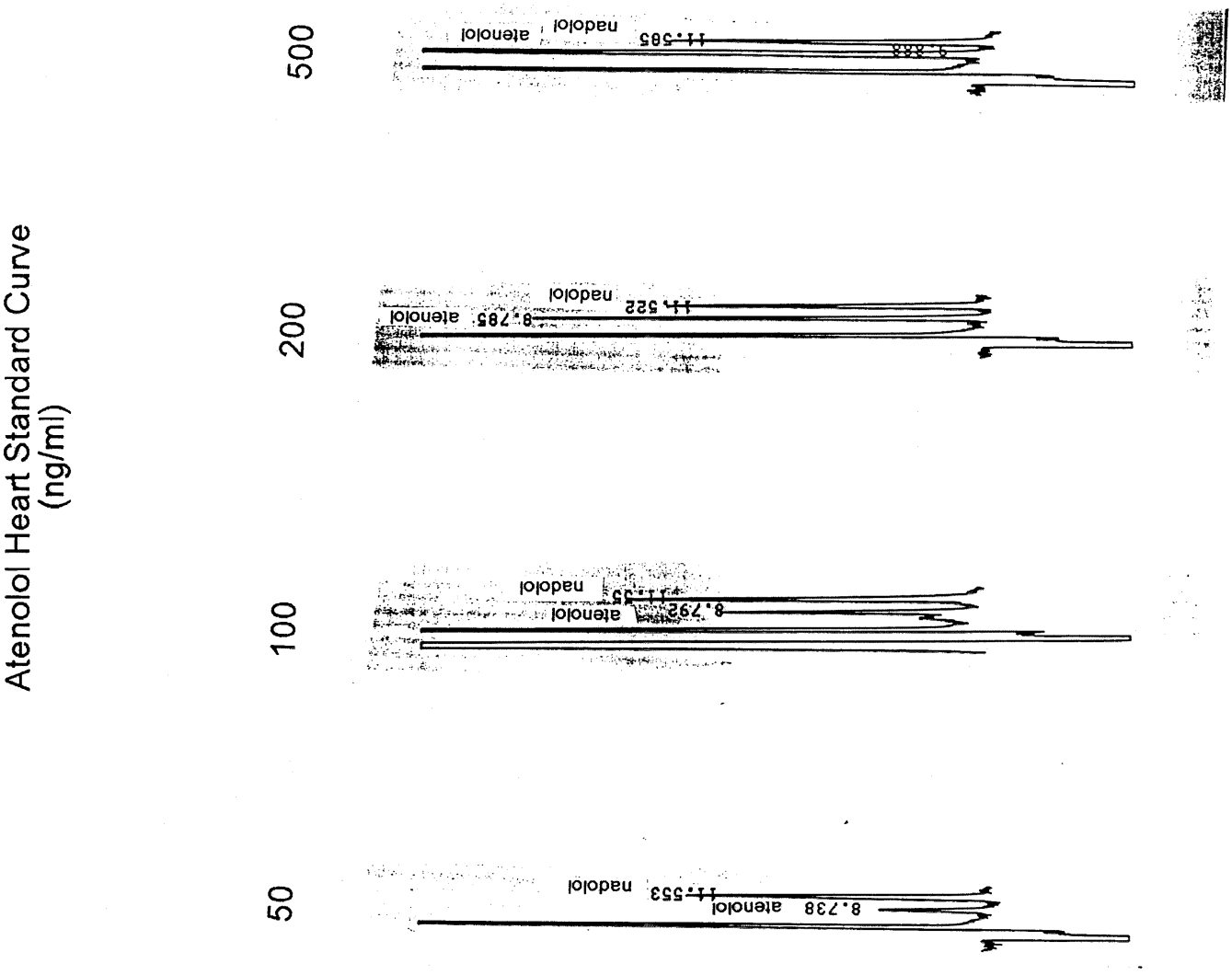


FIGURE 10. Standard Curve Chromatographs for Atenolol in Heart: Concentrations of 50-500 ng/ml Using Nadolol as Internal Standard

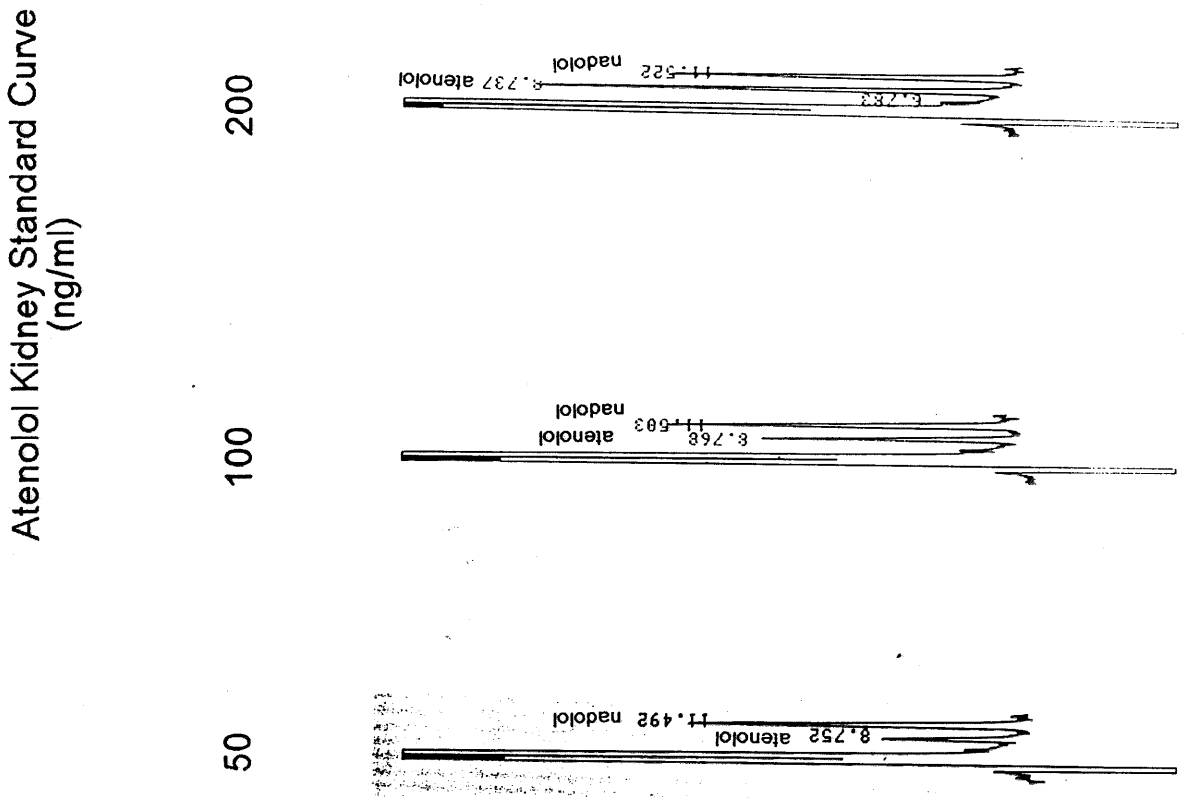


FIGURE 11. Standard Curve Chromatographs for Atenolol in Kidney: Concentrations of 50-500 ng/ml Using Nadolol as Internal Standard

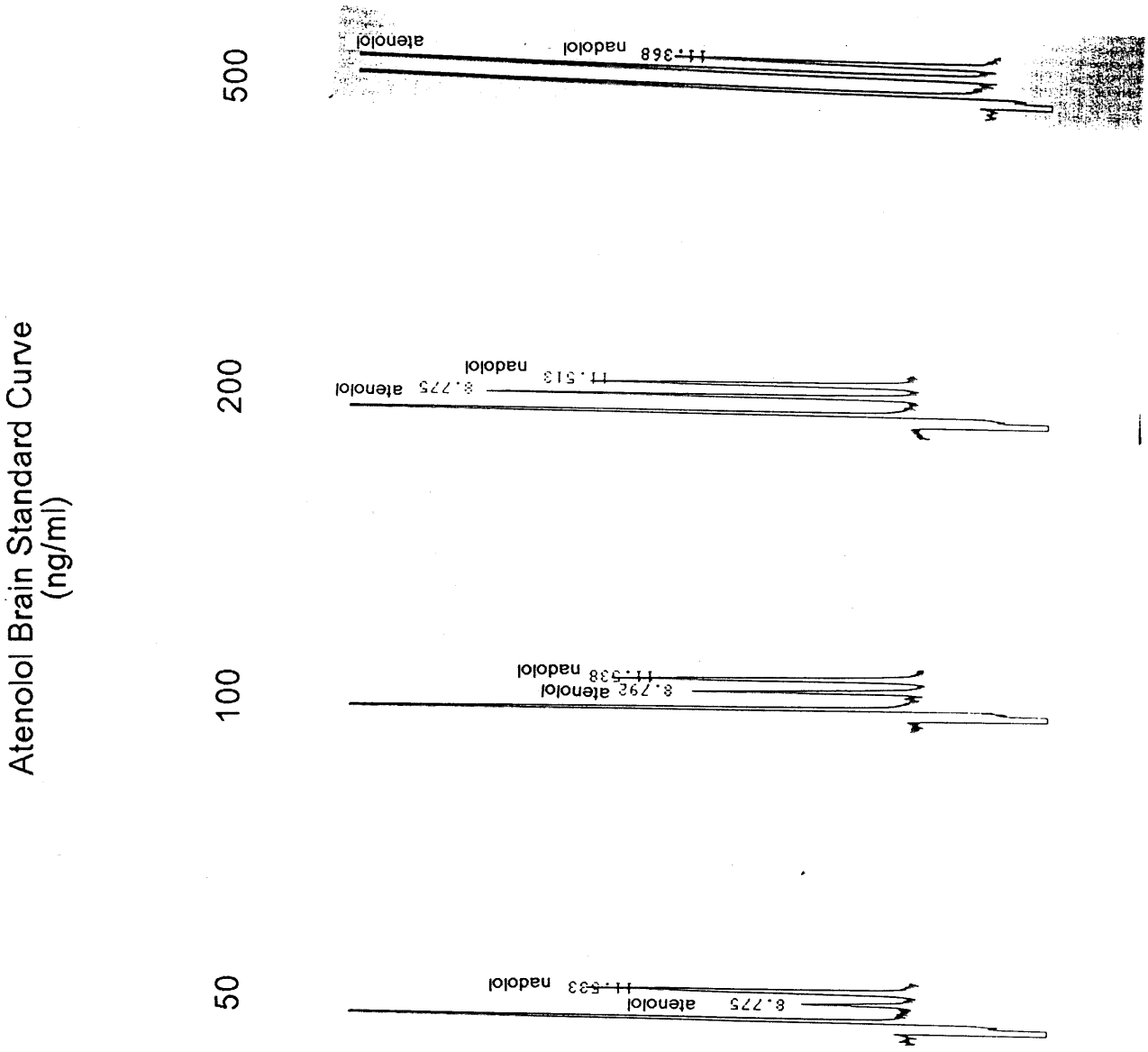


FIGURE 12. Standard Curve Chromatographs for Atenolol in Brain: Concentrations of 50-500 ng/ml Using Nadolol as Internal Standard

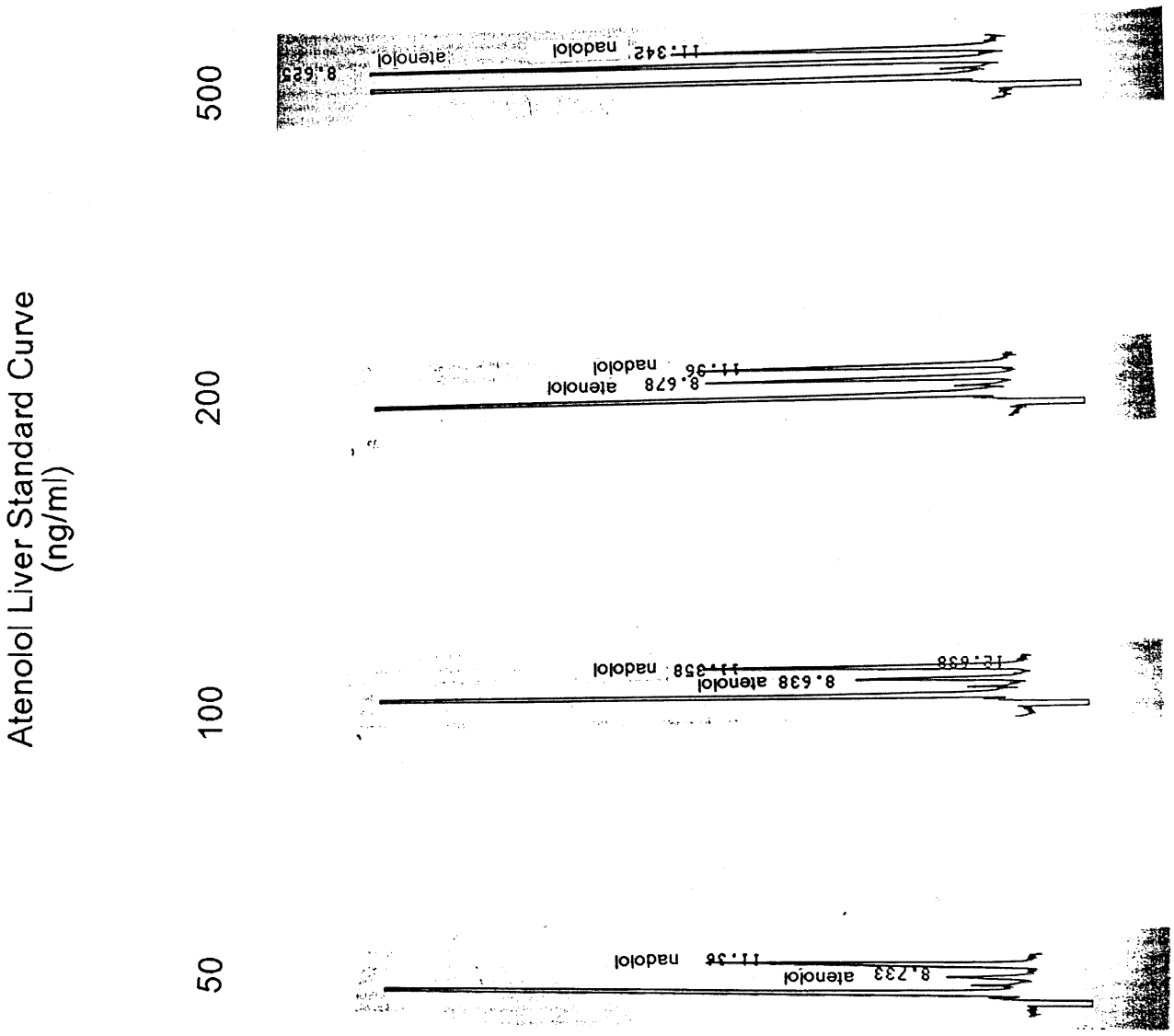


FIGURE 13. Standard Curve Chromatographs for Atenolol in Liver: Concentrations of 50-500 ng/ml Using Nadolol as Internal Standard

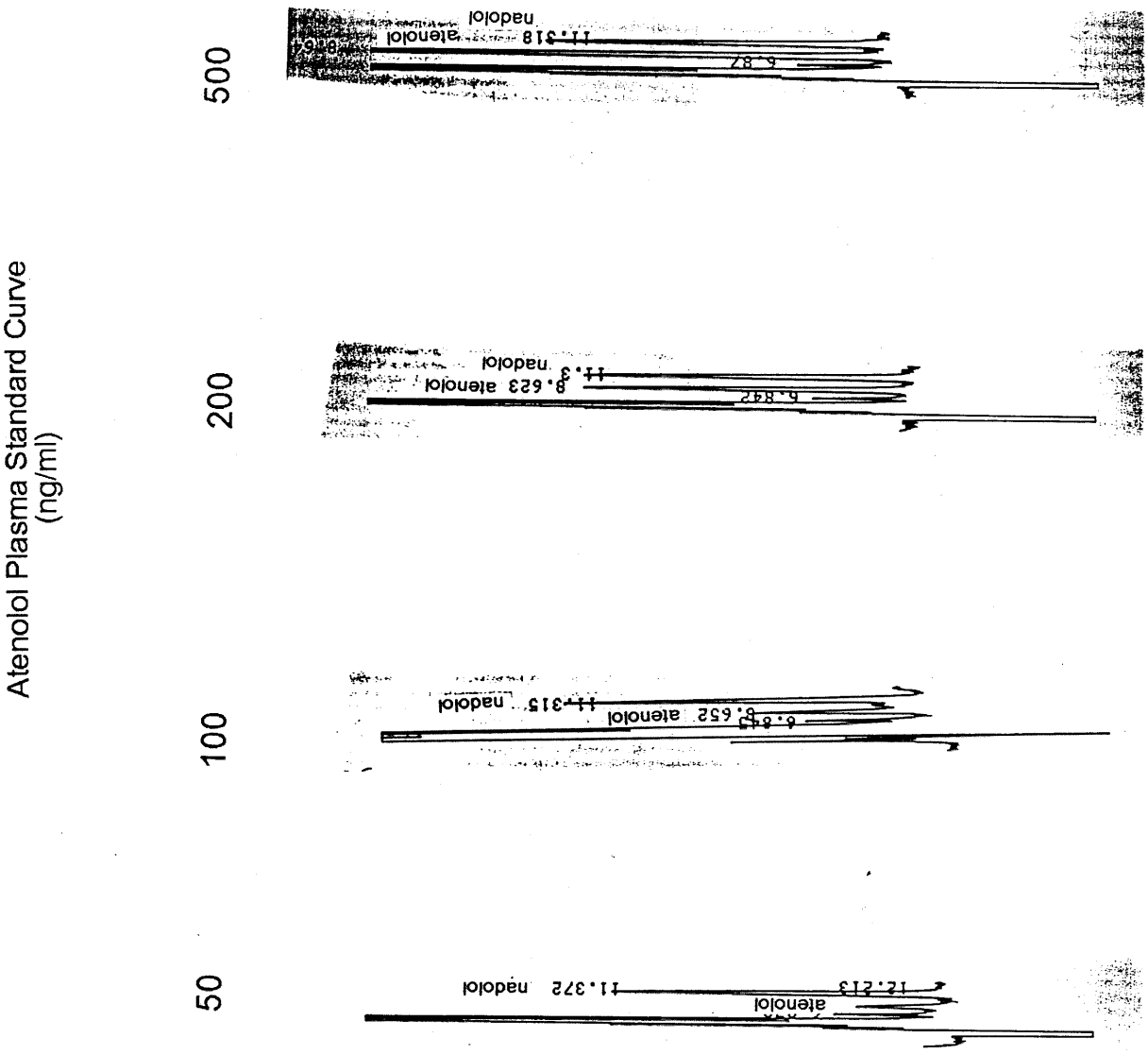


FIGURE 14. Standard Curve Chromatographs for Atenolol in Plasma: Concentrations of 50-500 ng/ml Using Nadolol as Internal Standard

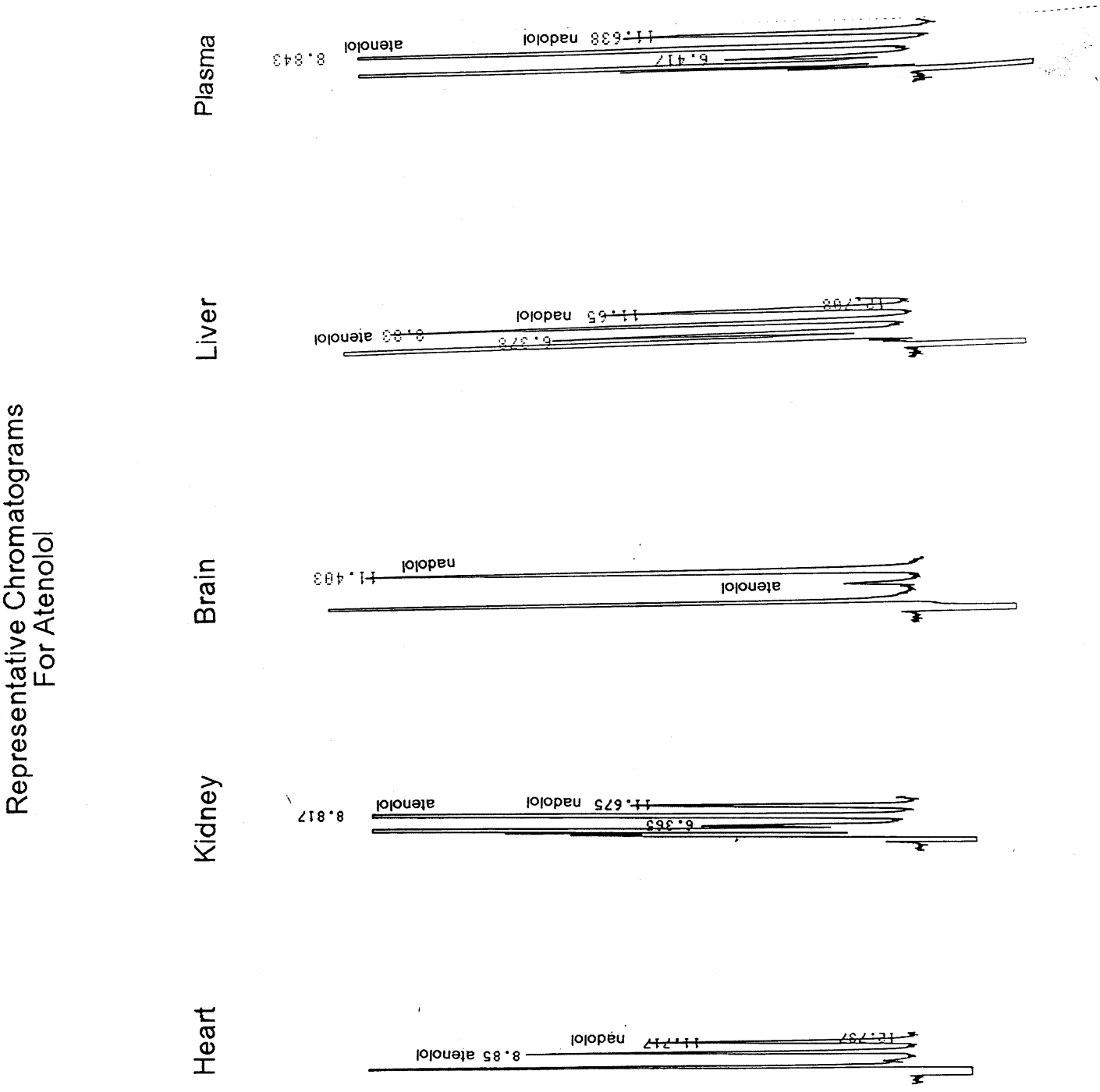


FIGURE 15. Representative Chromatograms for Atenolol in Heart, Kidney, Brain, Liver and Plasma Using Nadolol as Internal Standard

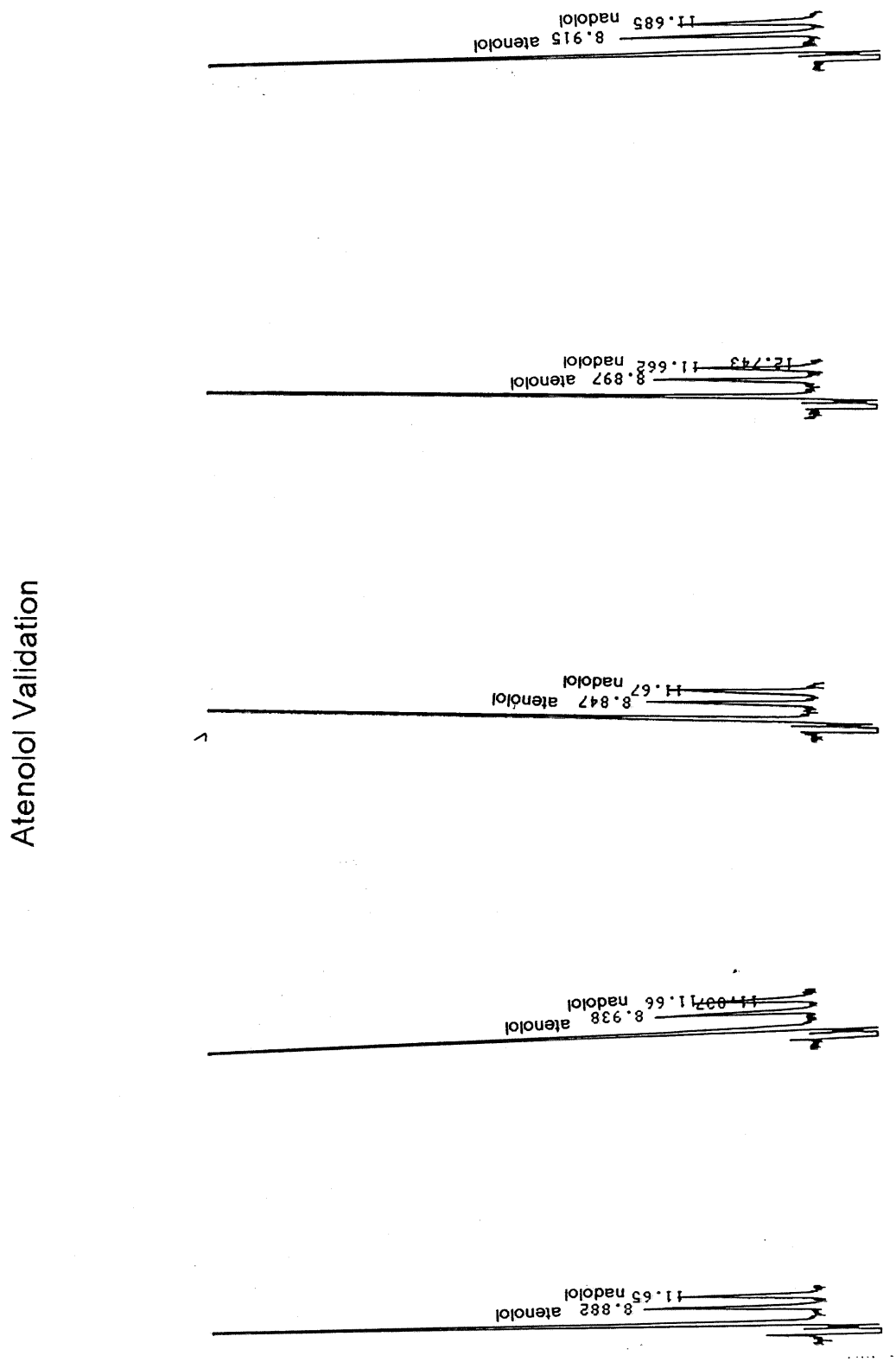


FIGURE 16. Representative Chromatograms for Atenolol Validation Using Nadolol as Internal Standard

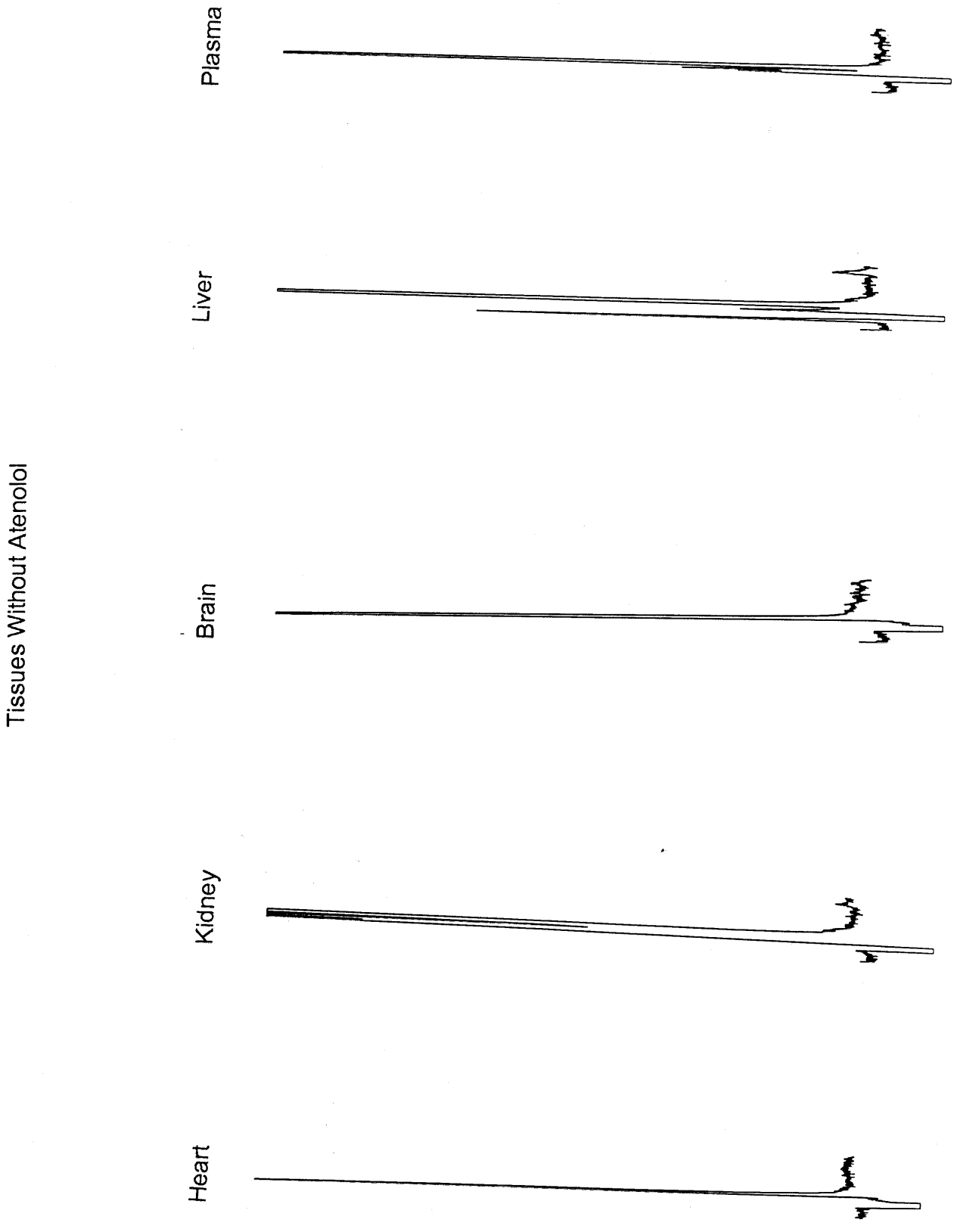


FIGURE 17. Representative Chromatograms for Heart, Kidney, Brain, Liver, and Plasma Extraction Without Atenolol

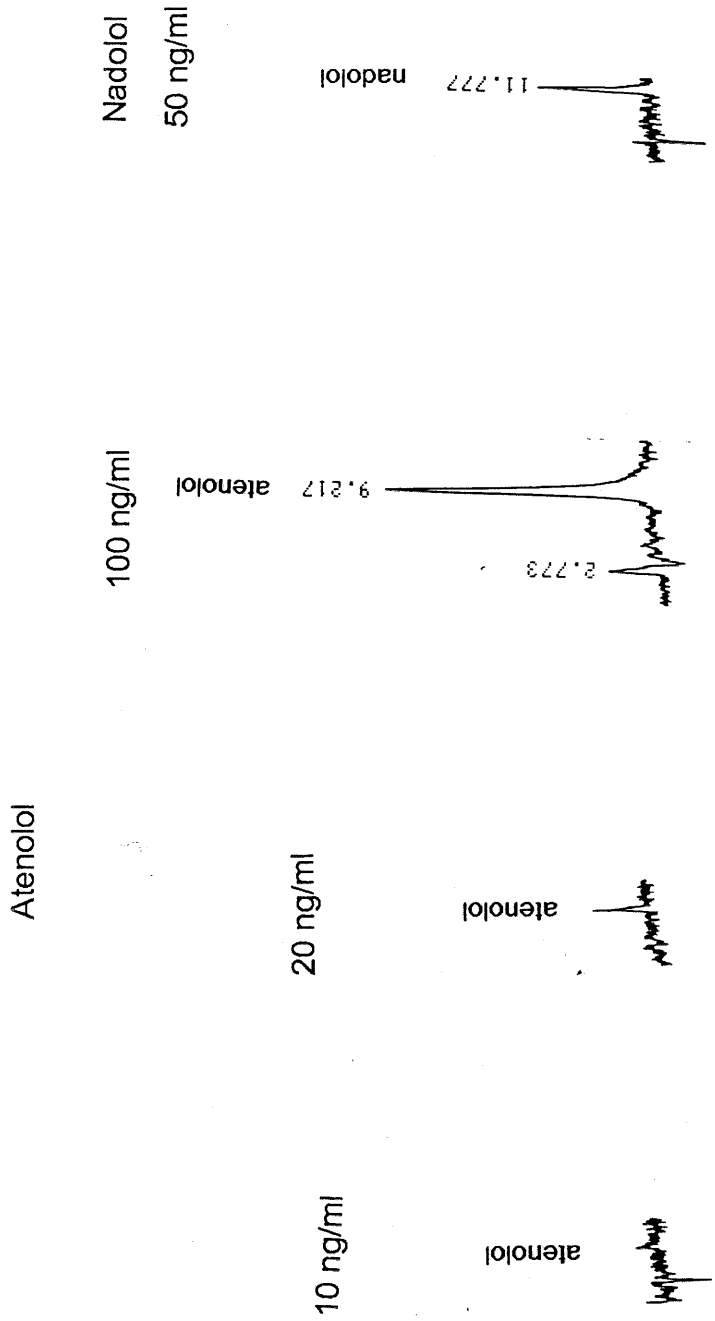


FIGURE 18. Representative Chromatograms of Atenolol Standards at 10, 20 and 100 ng/ml and Nadolol Standard at 50 ng/ml

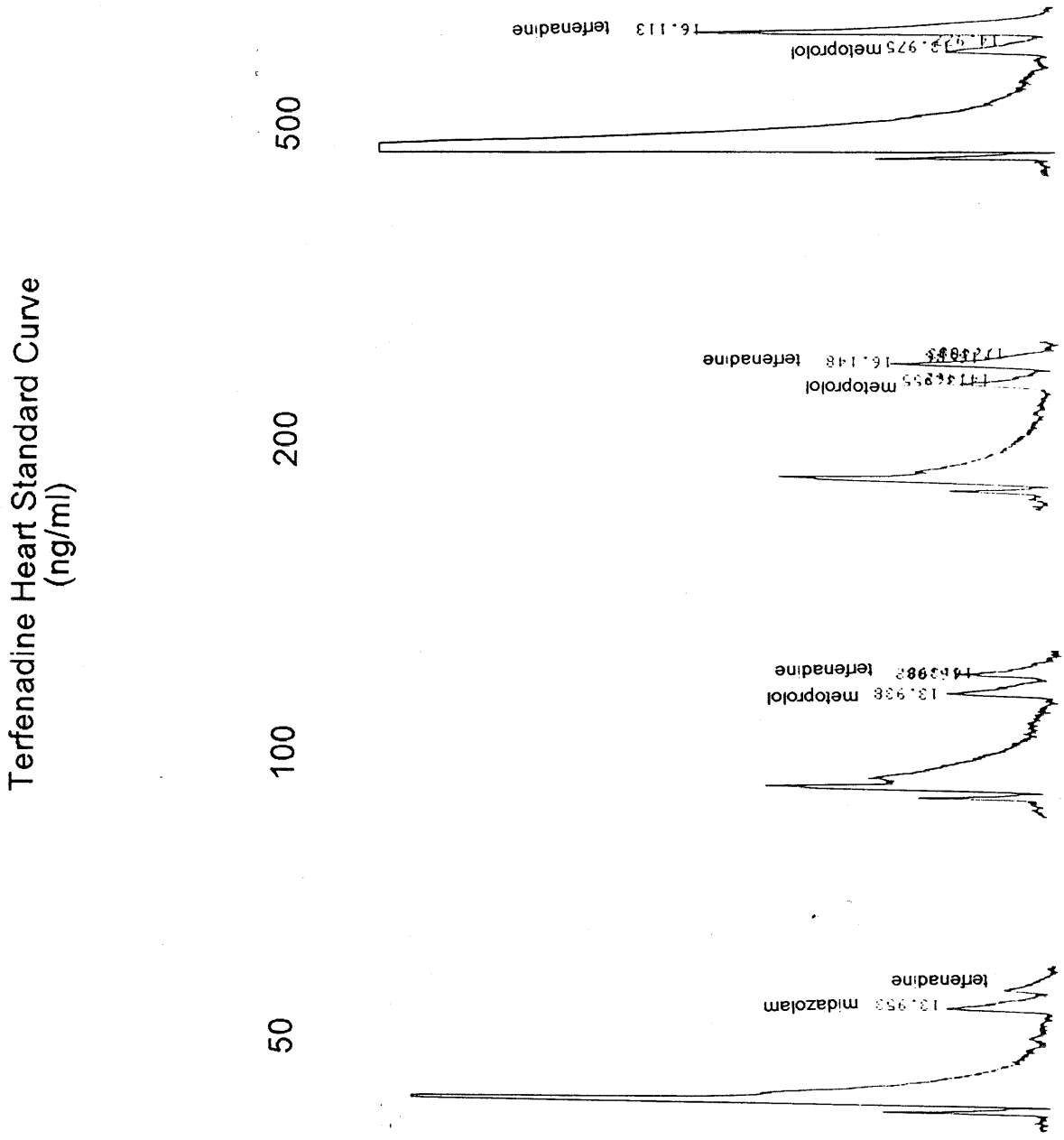


FIGURE 19. Standard Curve Chromatographs for Terfenadine in Heart: Concentrations of 50-500 ng/ml Using Metoprolol as Internal Standard

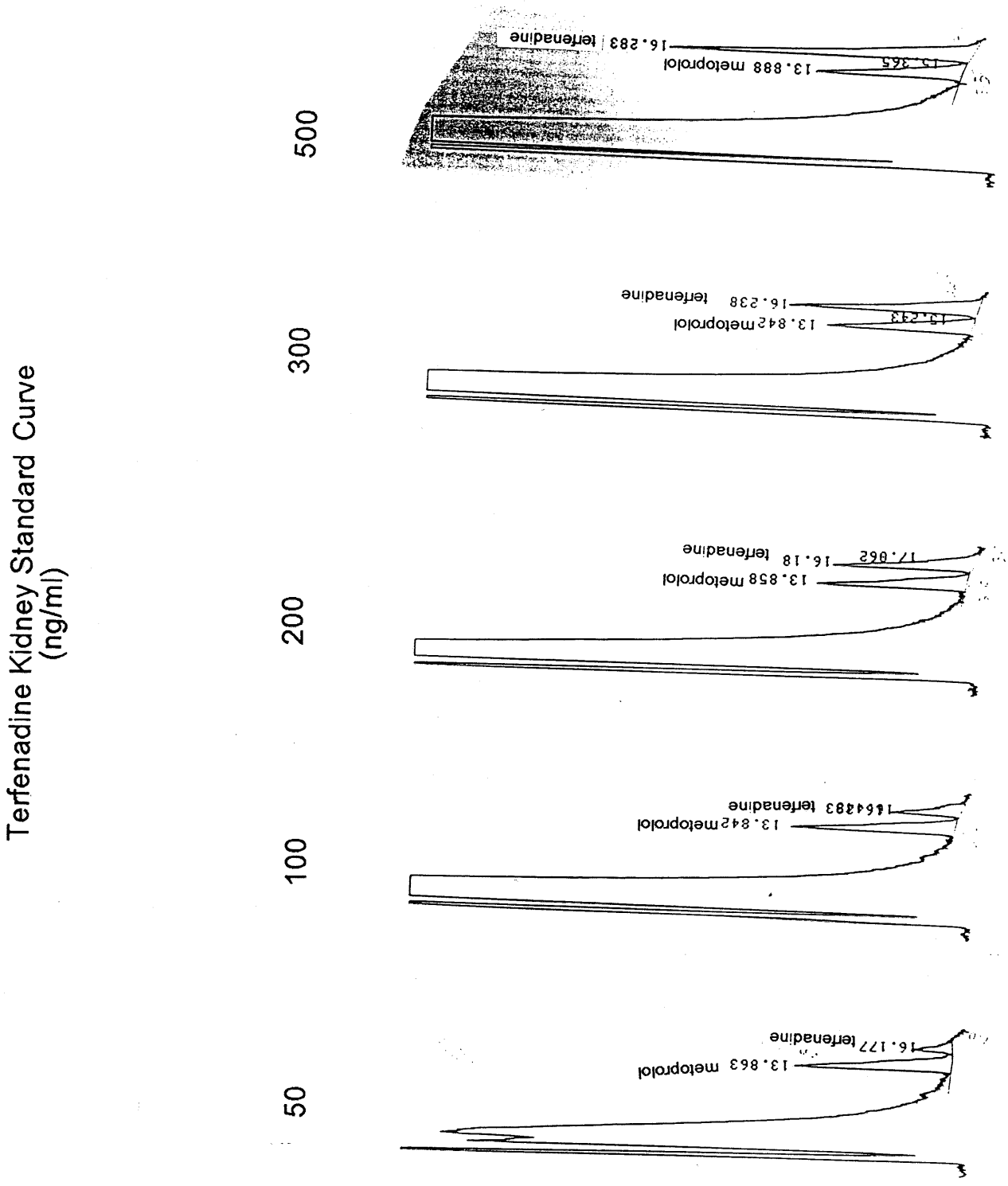


FIGURE 20. Standard Curve Chromatographs for Terfenadine in Kidney: Concentrations of 50-500 ng/ml Using Metoprolol as Internal Standard

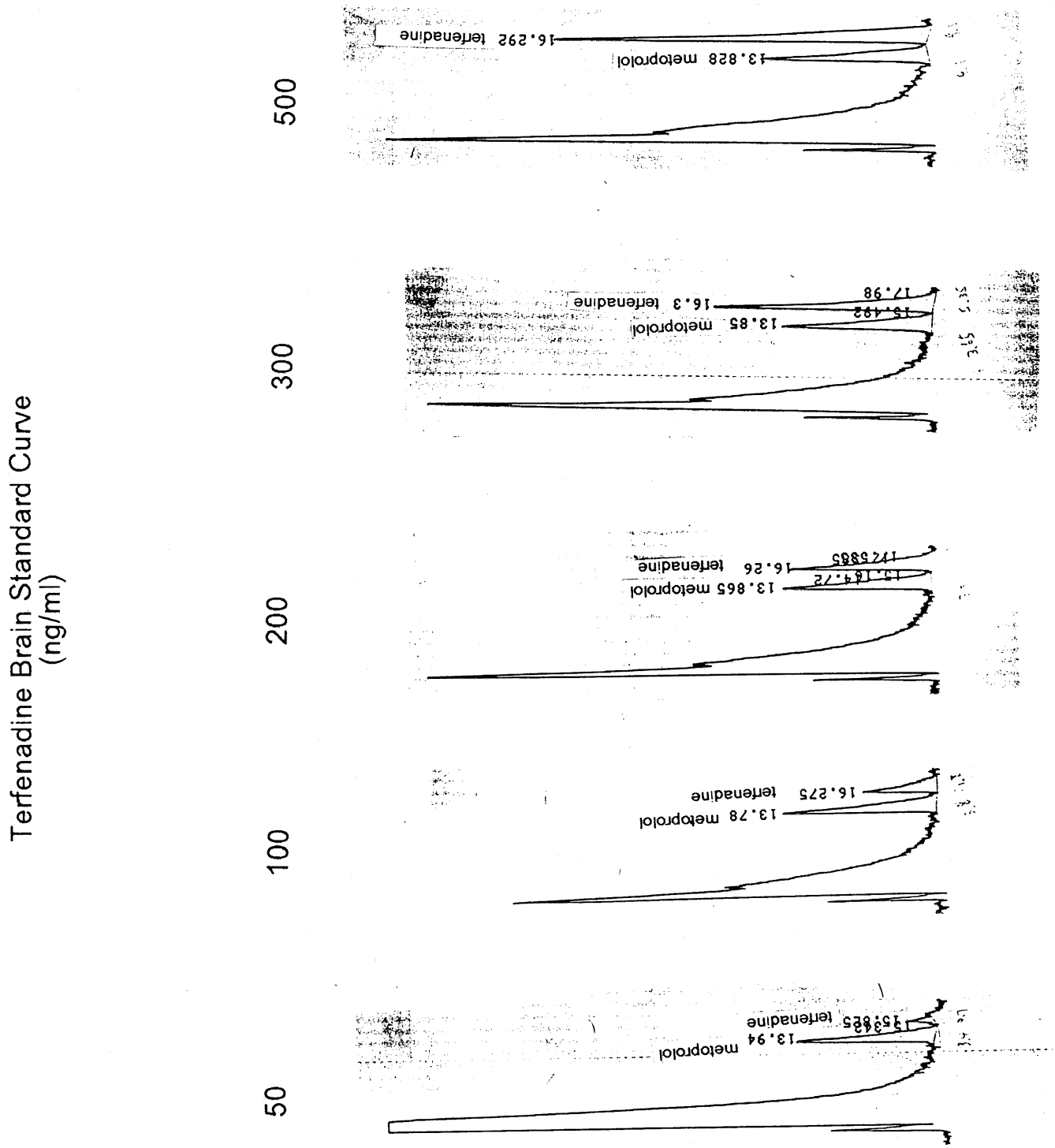


FIGURE 21. Standard Curve Chromatographs for Terfenadine in Brain: Concentrations of 50-500 ng/ml Using Metoprolol as Internal Standard

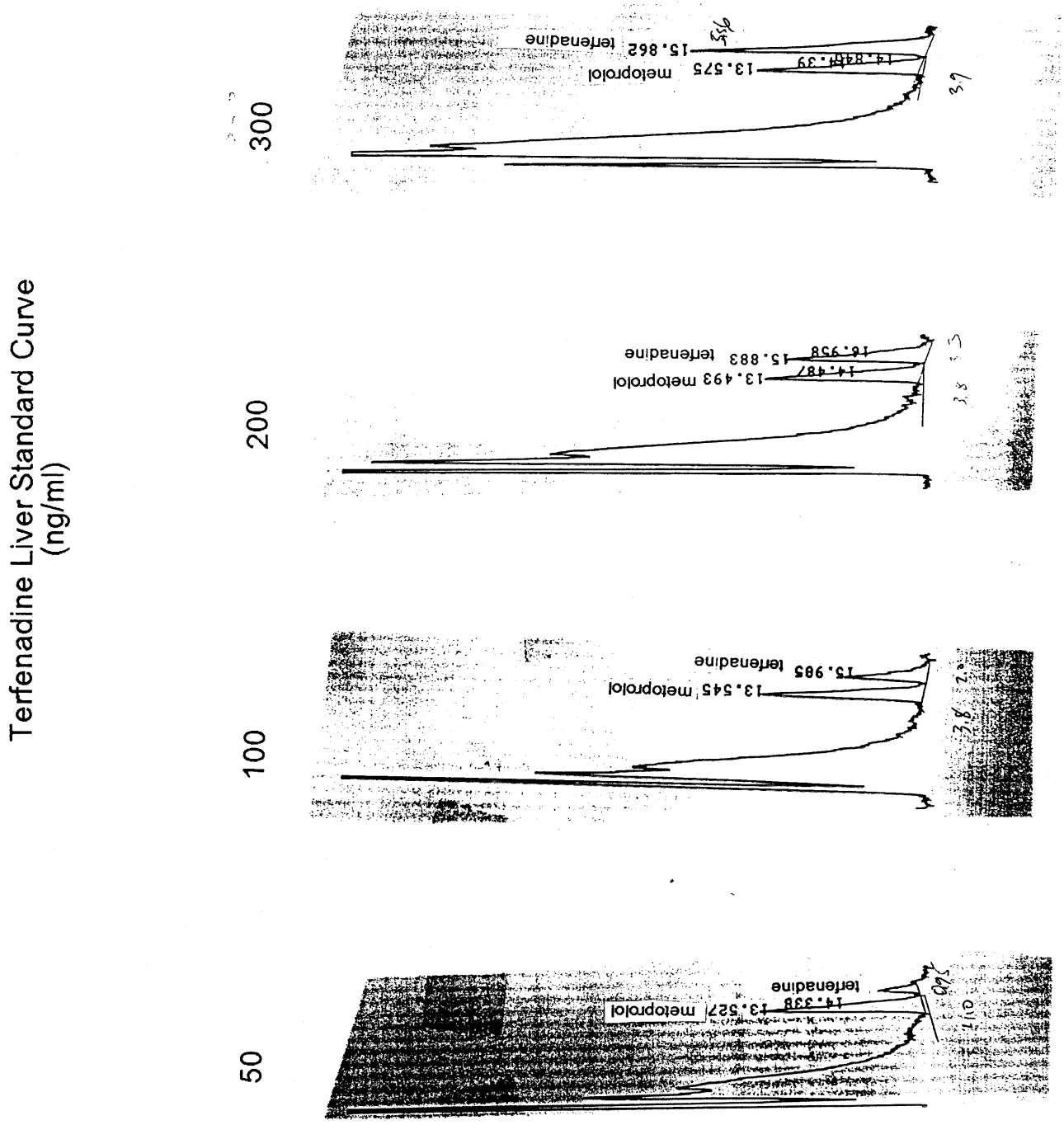


FIGURE 22. Standard Curve Chromatographs for Terfenadine in Liver: Concentrations of 50-300 ng/ml Using Metoprolol as Internal Standard

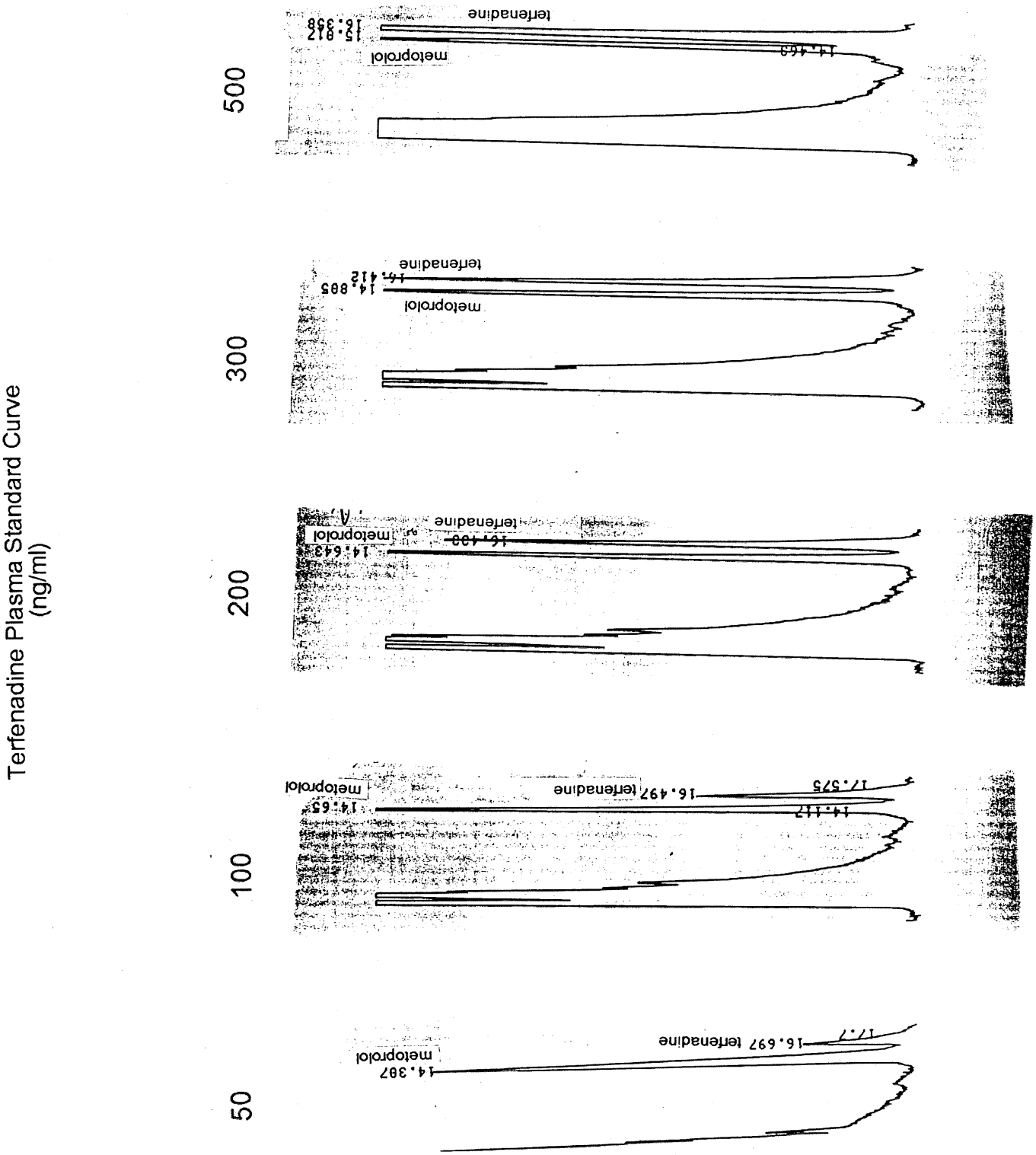


FIGURE 23. Standard Curve Chromatographs for Terfenadine in Plasma: Concentrations of 50-500 ng/ml Using Metoprolol as Internal Standard

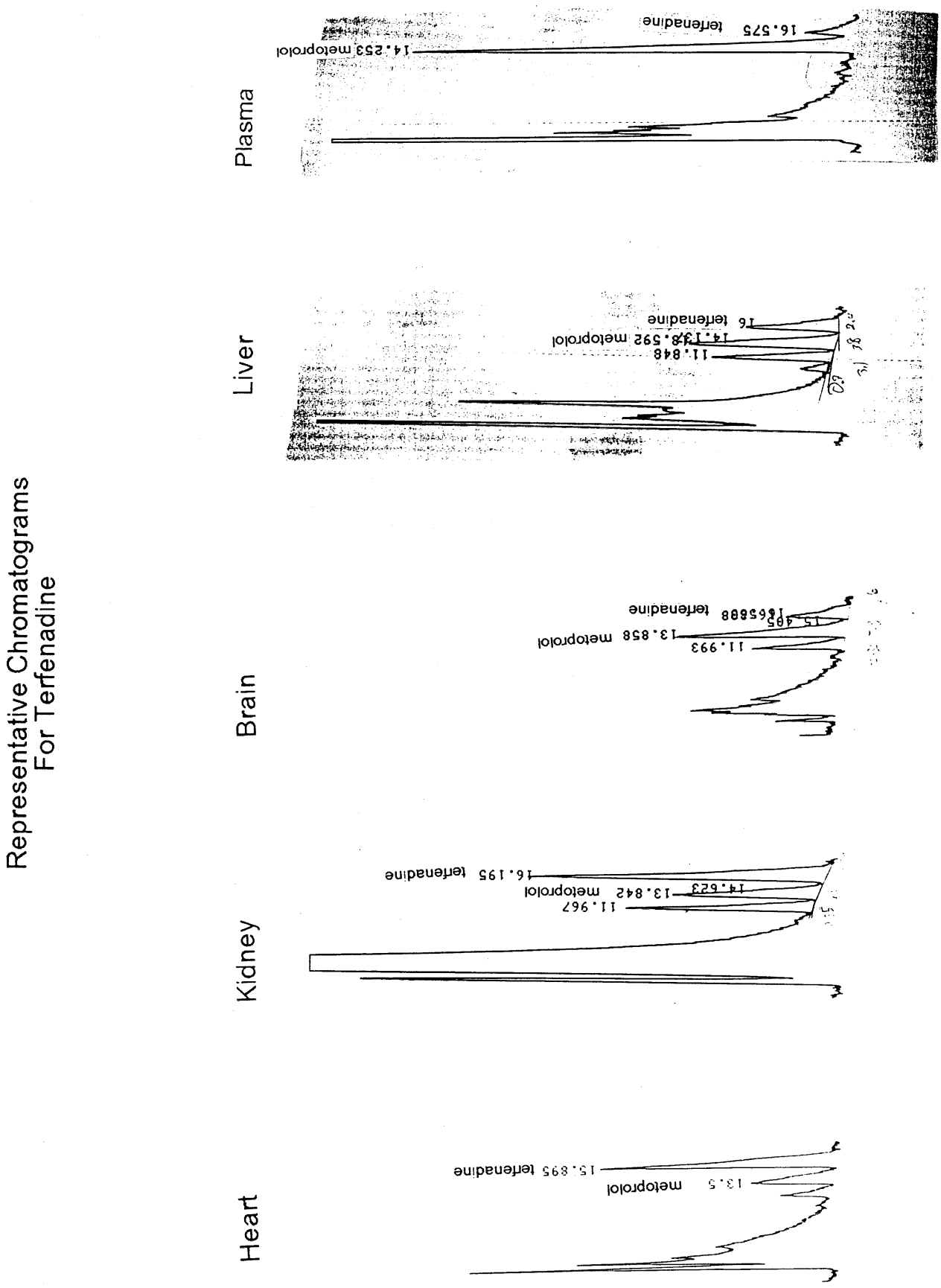


FIGURE 24. Representative Chromatograms for Terfenadine in Heart, Kidney, Brain, Liver and Plasma Using Metoprolol as Internal Standard

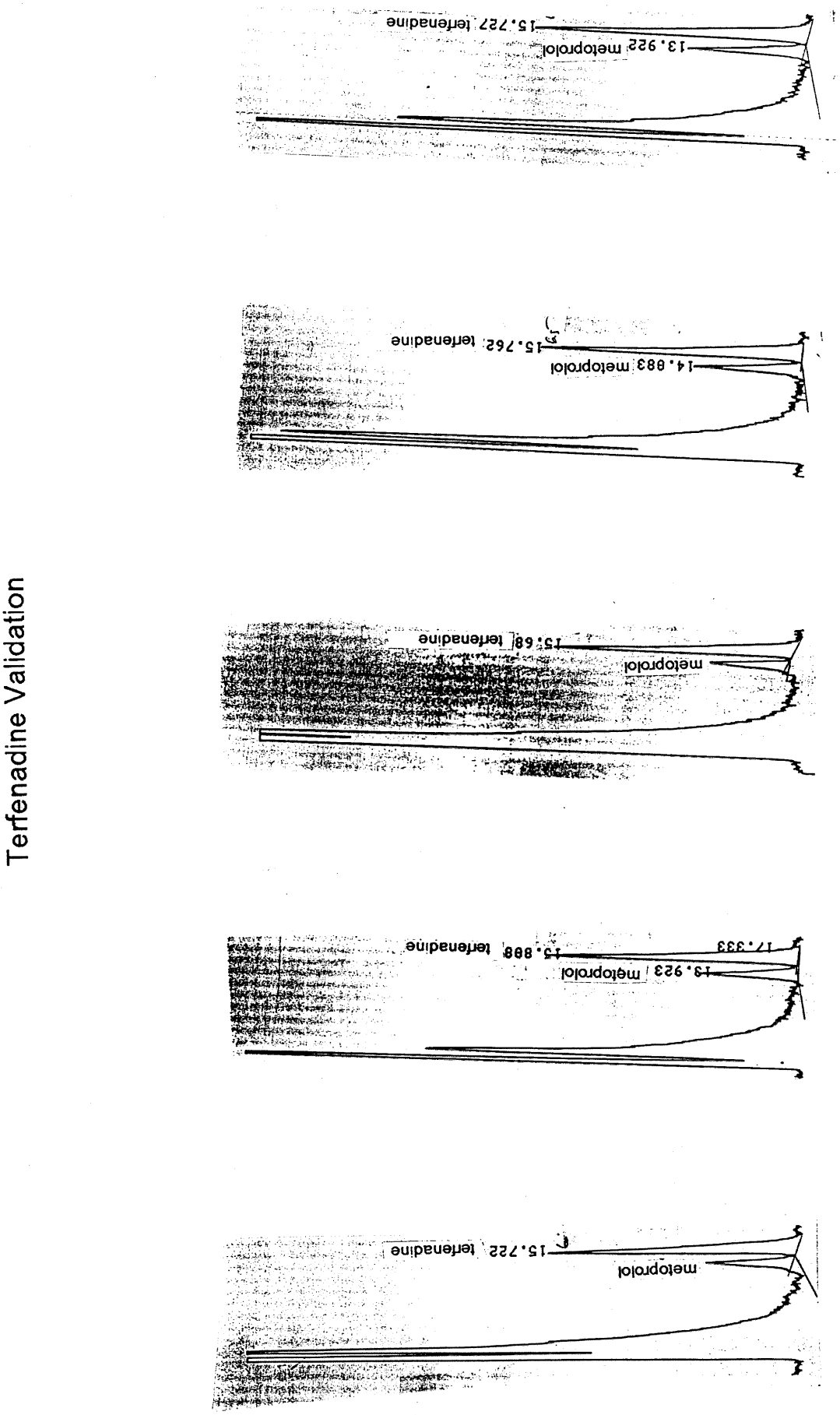


FIGURE 25. Representative Chromatograms for Terfenadine Validation Using Metoprolol as Internal Standard

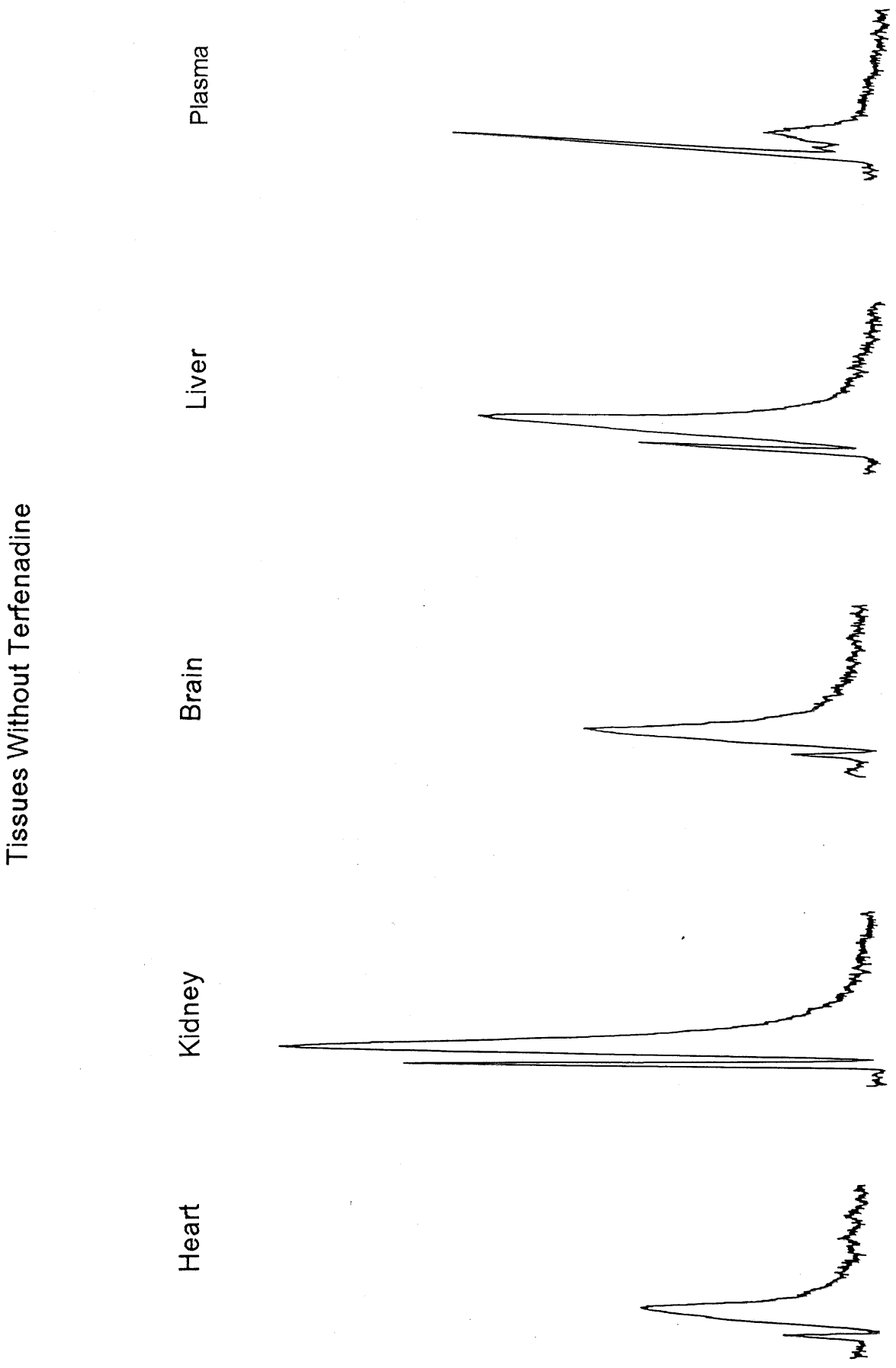


FIGURE 26. Represenative Chromatograms for Heart, Kidney, Brain, Liver, and Plasma Extraction Without Terfenadine

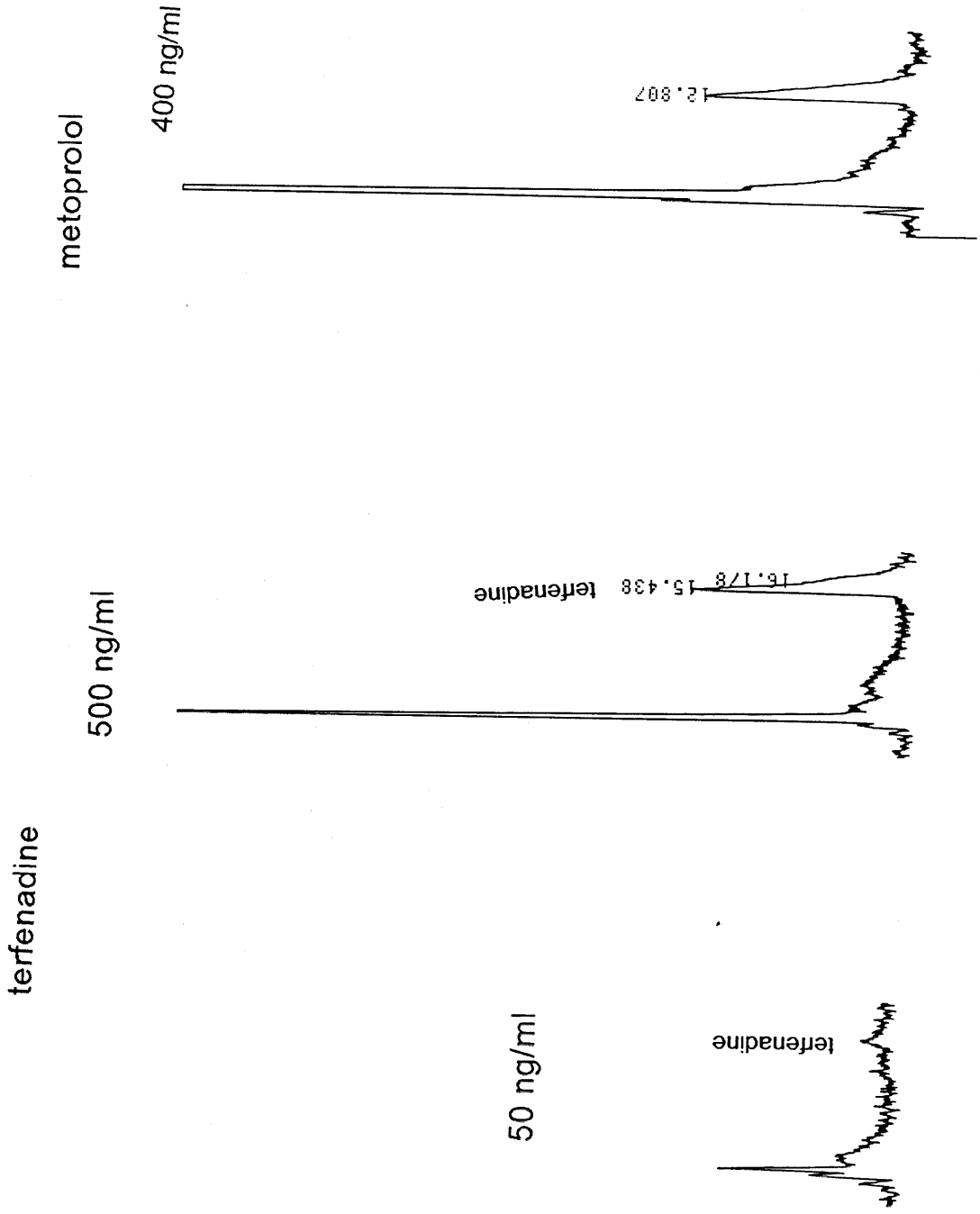


FIGURE 27. Representative Chromatograms of Terfenadine Standards at 50 and 500 ng/ml and Metoprolol Standard at 400 ng/ml

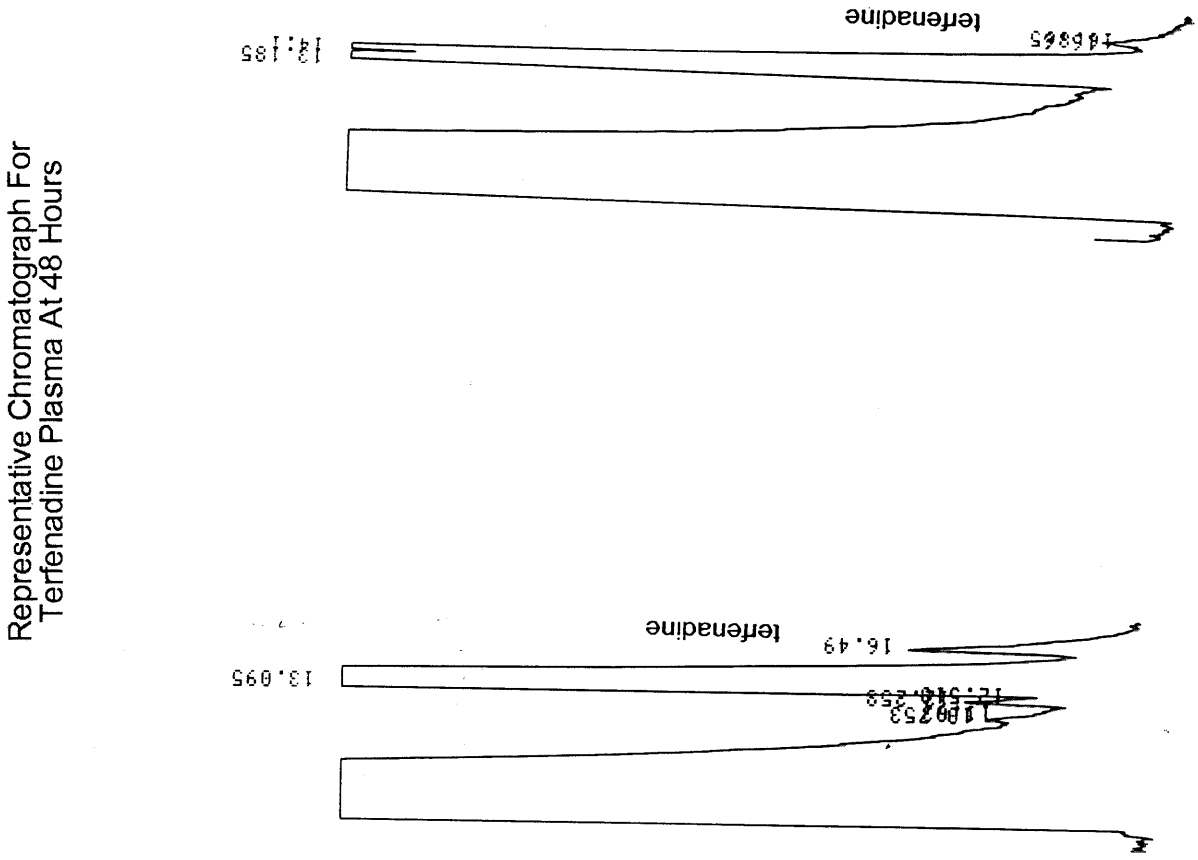
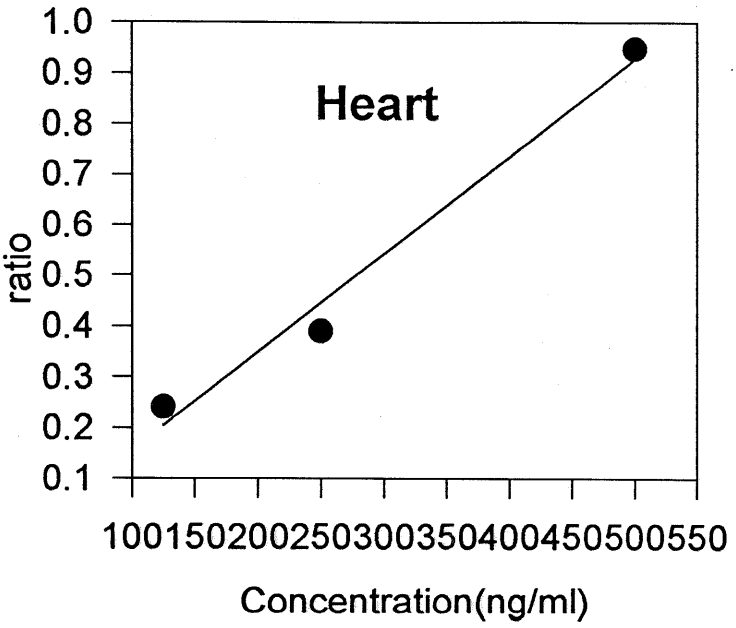
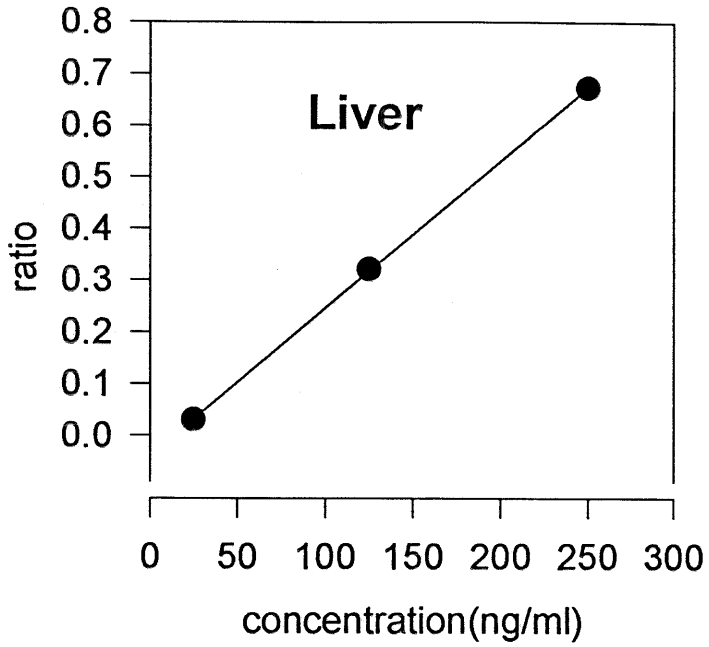
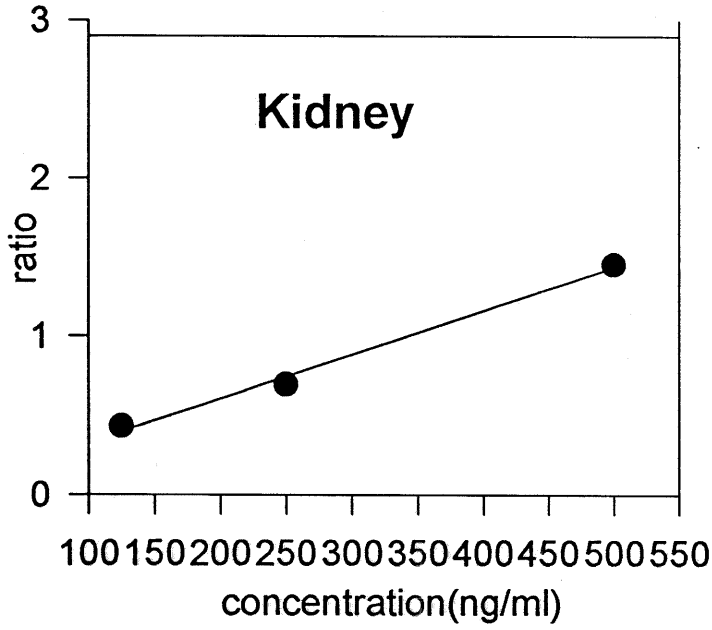
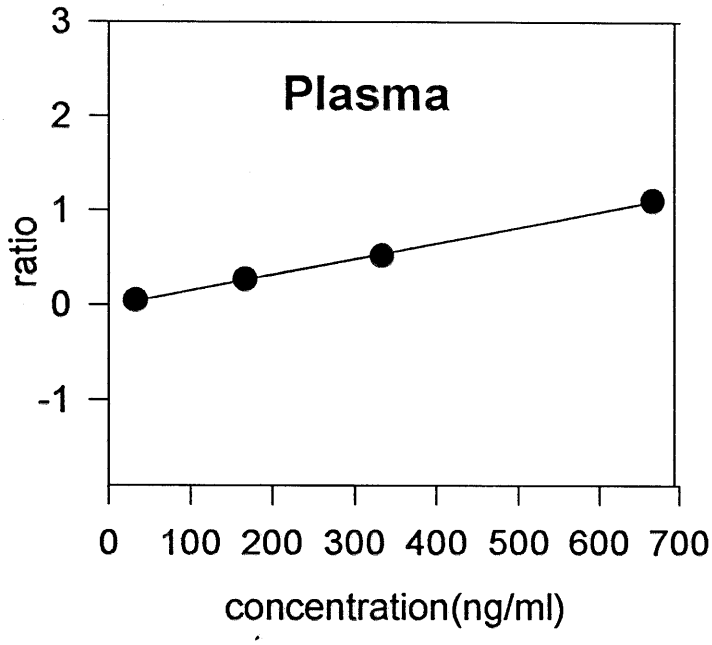


FIGURE 28. Representative Chromatograms of Postmortem Terfenadine Level in Plasma at 48 Hours









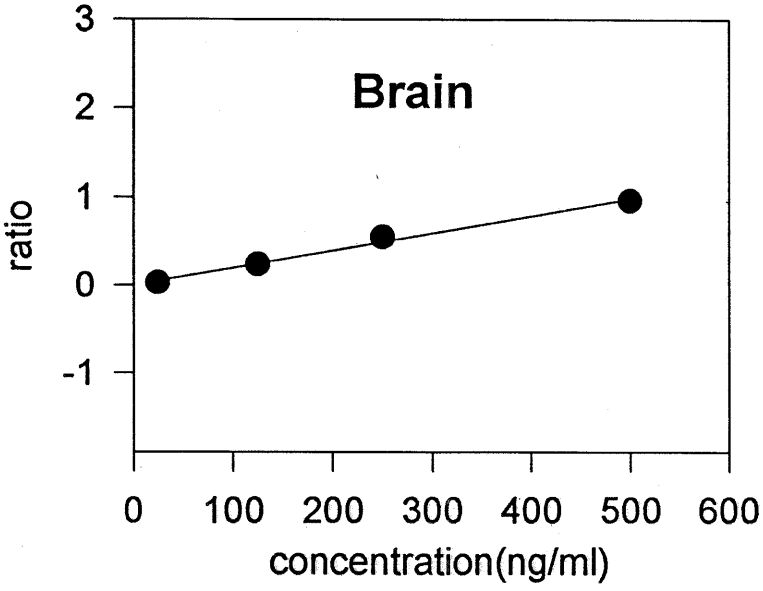


FIGURE 29. Graphs of standard curve (concentration vs. the ratio of drug/internal standard) for alprazolam in heart, kidney, brain, liver, and plasma

FIGURE 30. Alprazolam concentration in plasma (ng/ml, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

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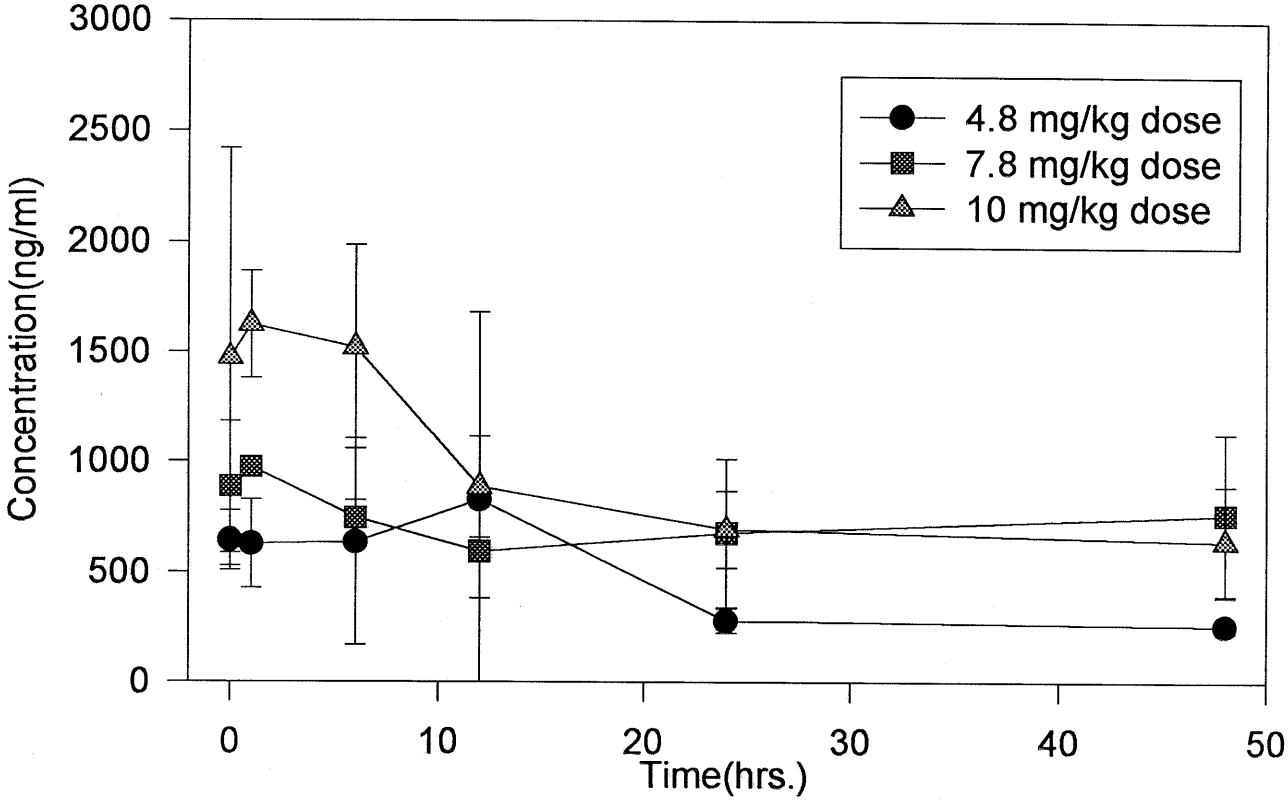
ALPRAZOLAM CONCENTRATION IN PLASMA (NG/ML)



ALPRAZOLAM CONCENTRATION IN PLASMA (NG/ML)Regression Order:2

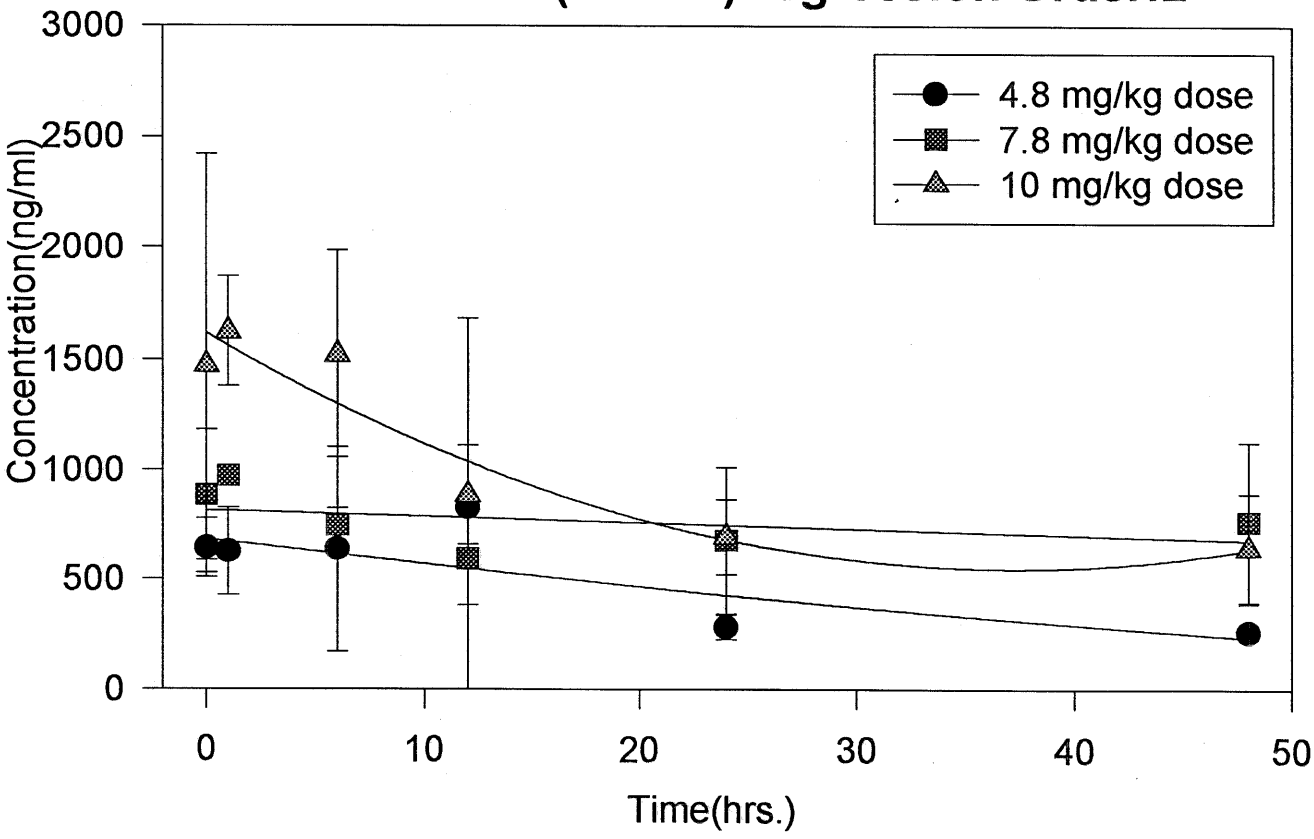


FIGURE 31. Alprazolam concentration in brain (ng/g, mean ± S.D., n=3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

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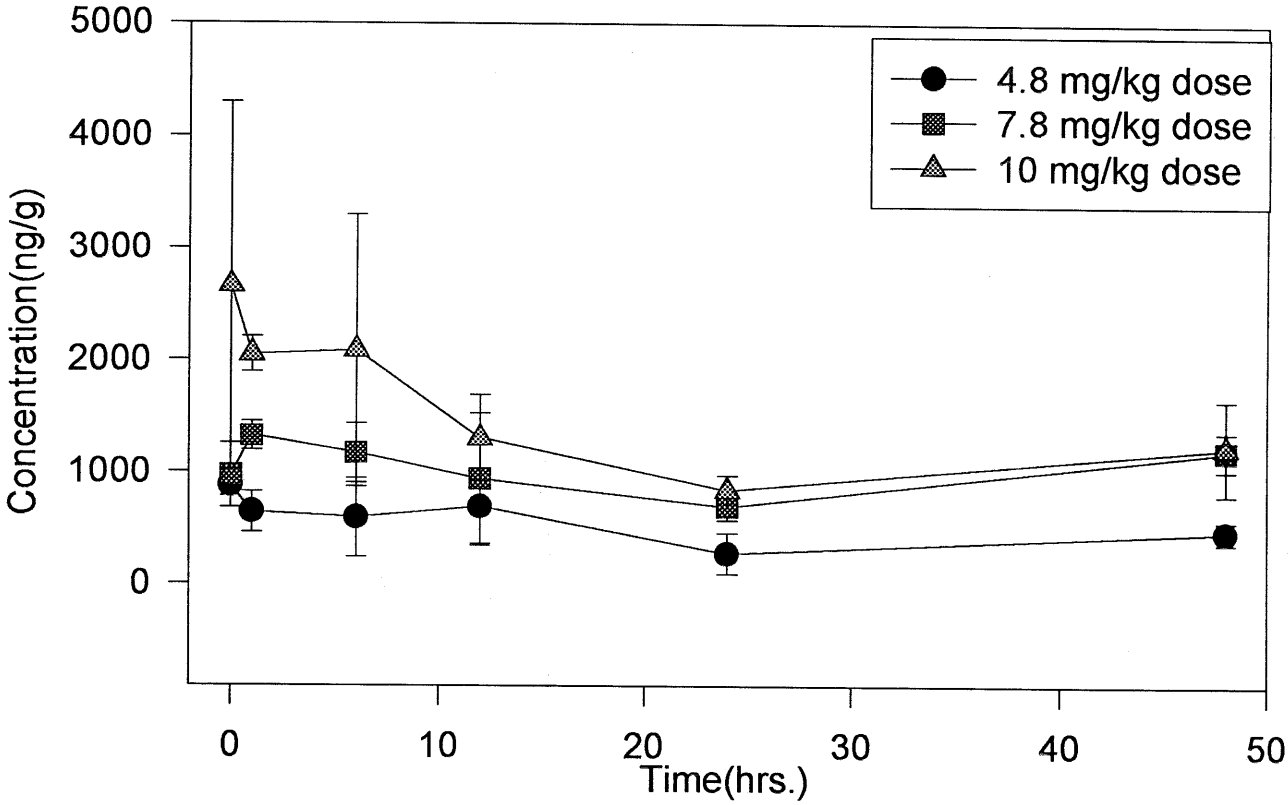
ALPRAZOLAM CONCENTRATION IN BRAIN (NG/G)



ALPRAZOLAM CONCENTRATION IN BRAIN (NG/G)Regression Order:2

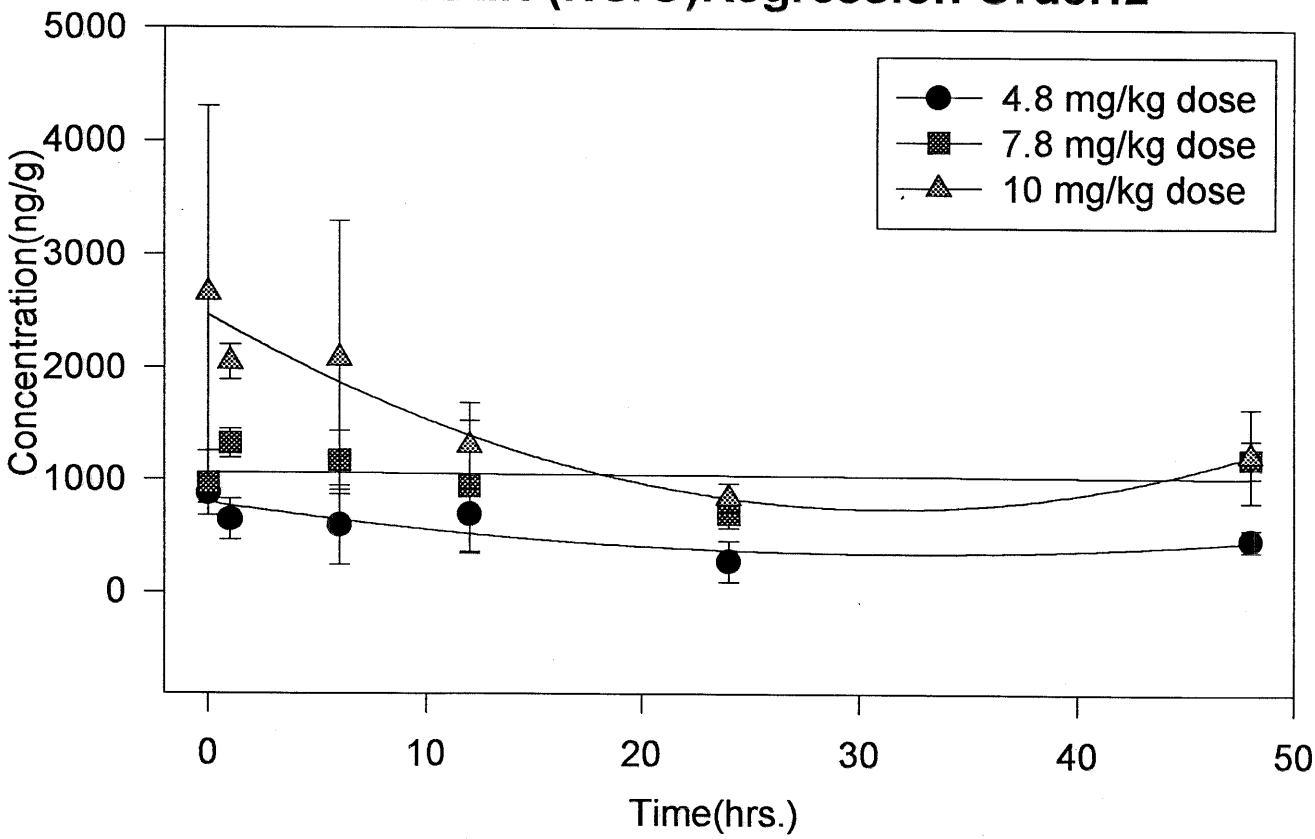


FIGURE 32. Alprazolam concentration in heart (ng/g, mean ± S.D., n=3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

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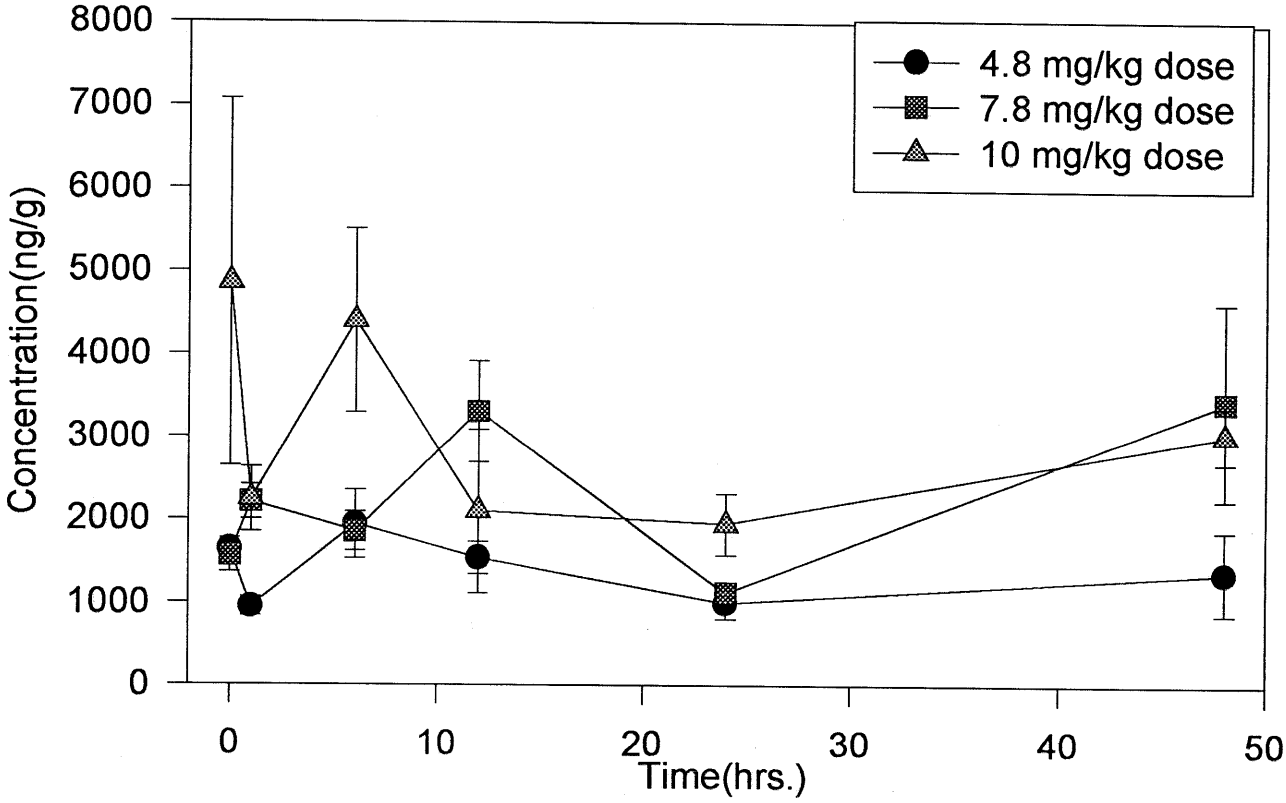
ALPRAZOLAM CONCENTRATION IN HEART (NG/G)



ALPRAZOLAM CONCENTRATION IN HEART (NG/G)Regression Order:2

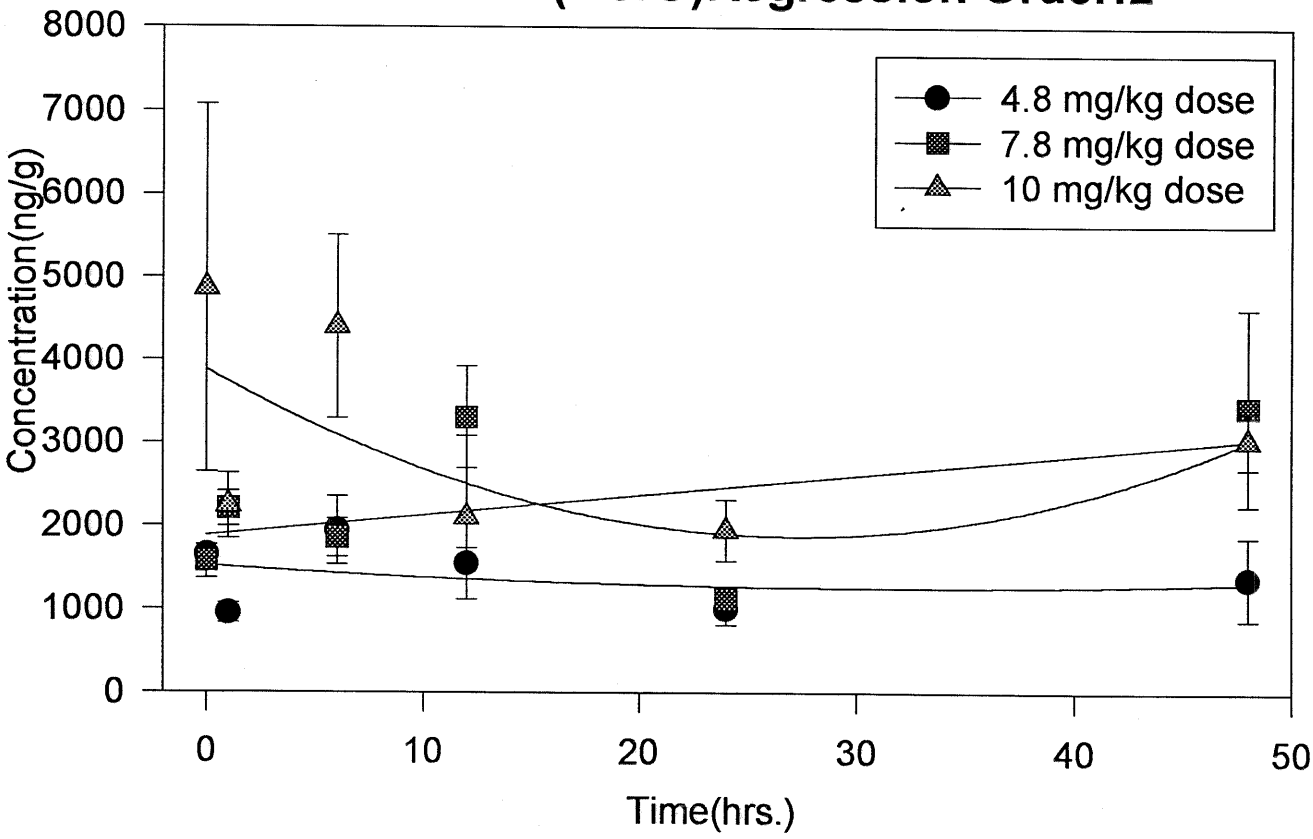


FIGURE 33. Alprazolam concentration in kidney (ng/g, mean ± S.D., n=3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

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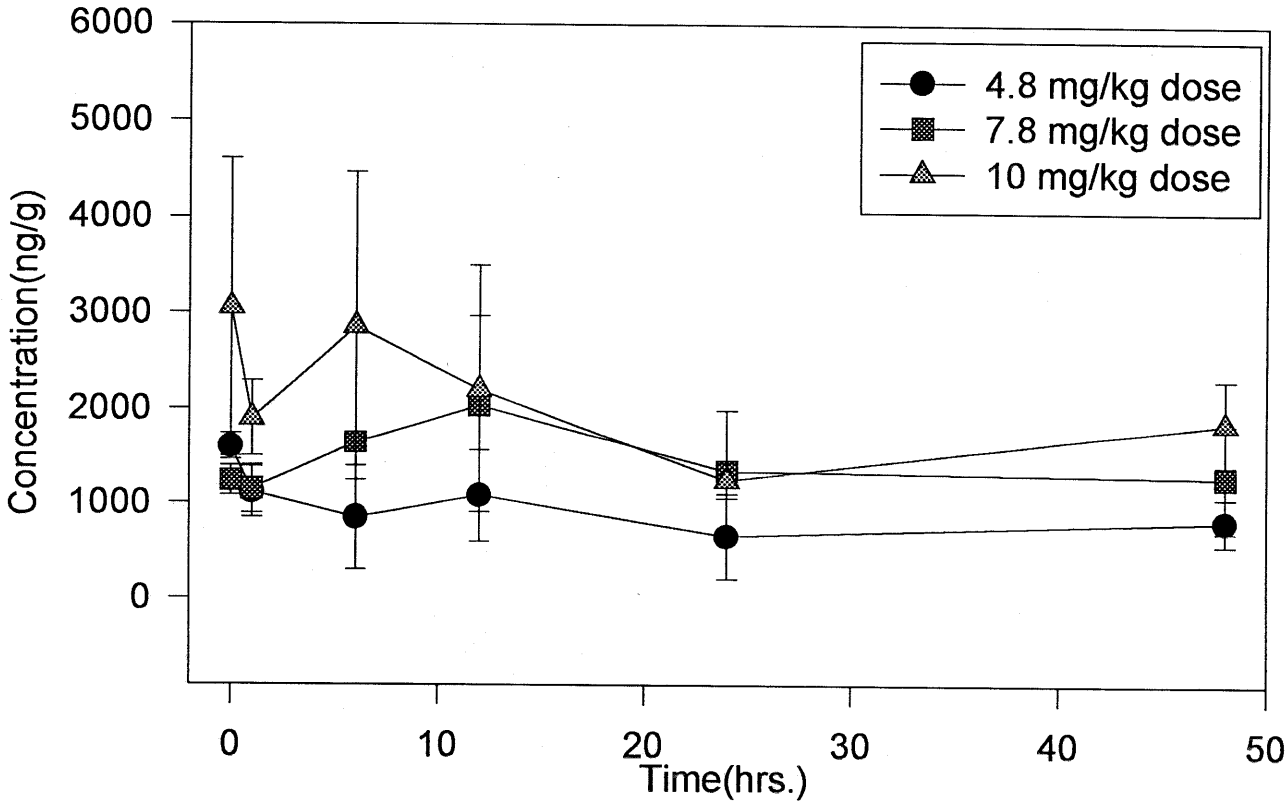
ALPRAZOLAM CONCENTRATION IN KIDNEY (NG/G)



ALPRAZOLAM CONCENTRATION IN KIDNEY (NG/G)Regression Order:2

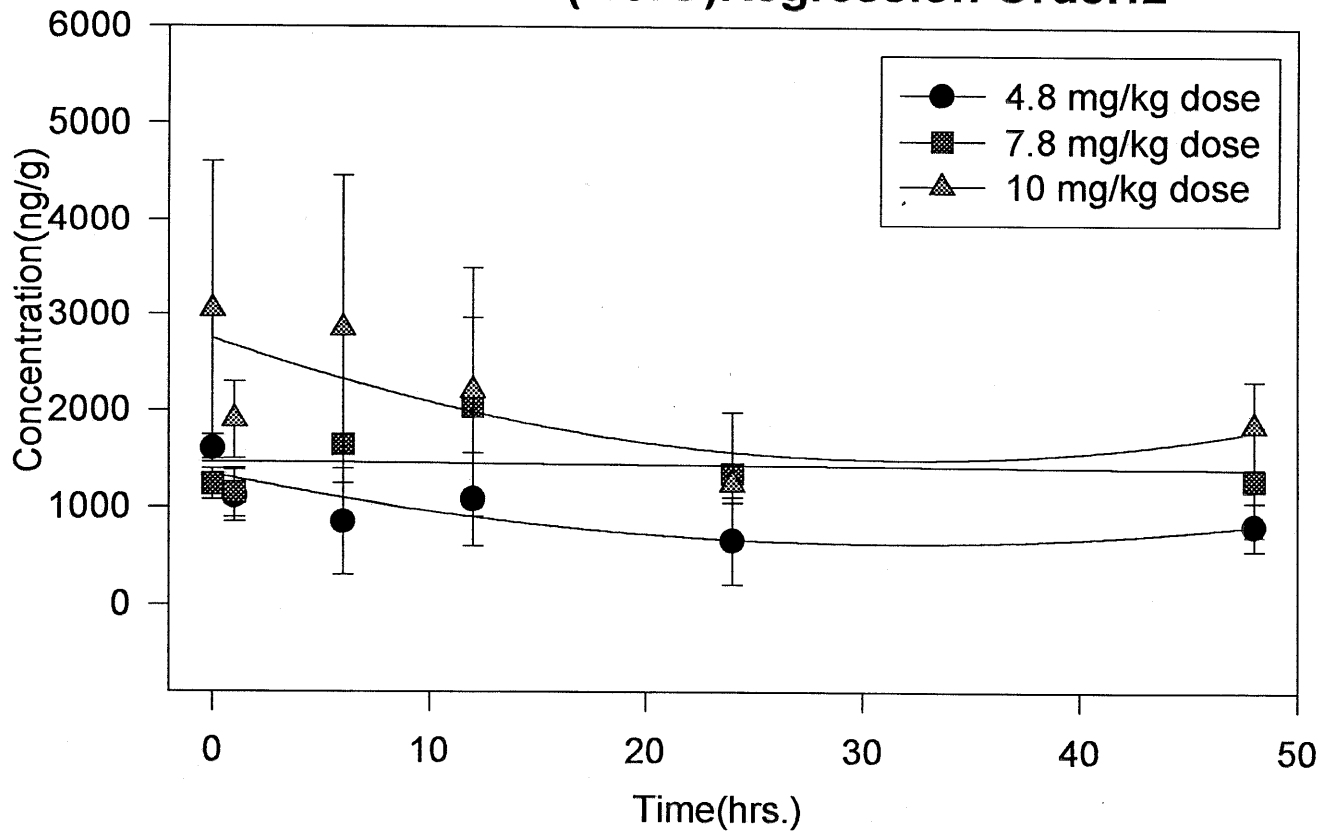


FIGURE 34. Alprazolam concentration in liver (ng/g, mean ± S.D., n=3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

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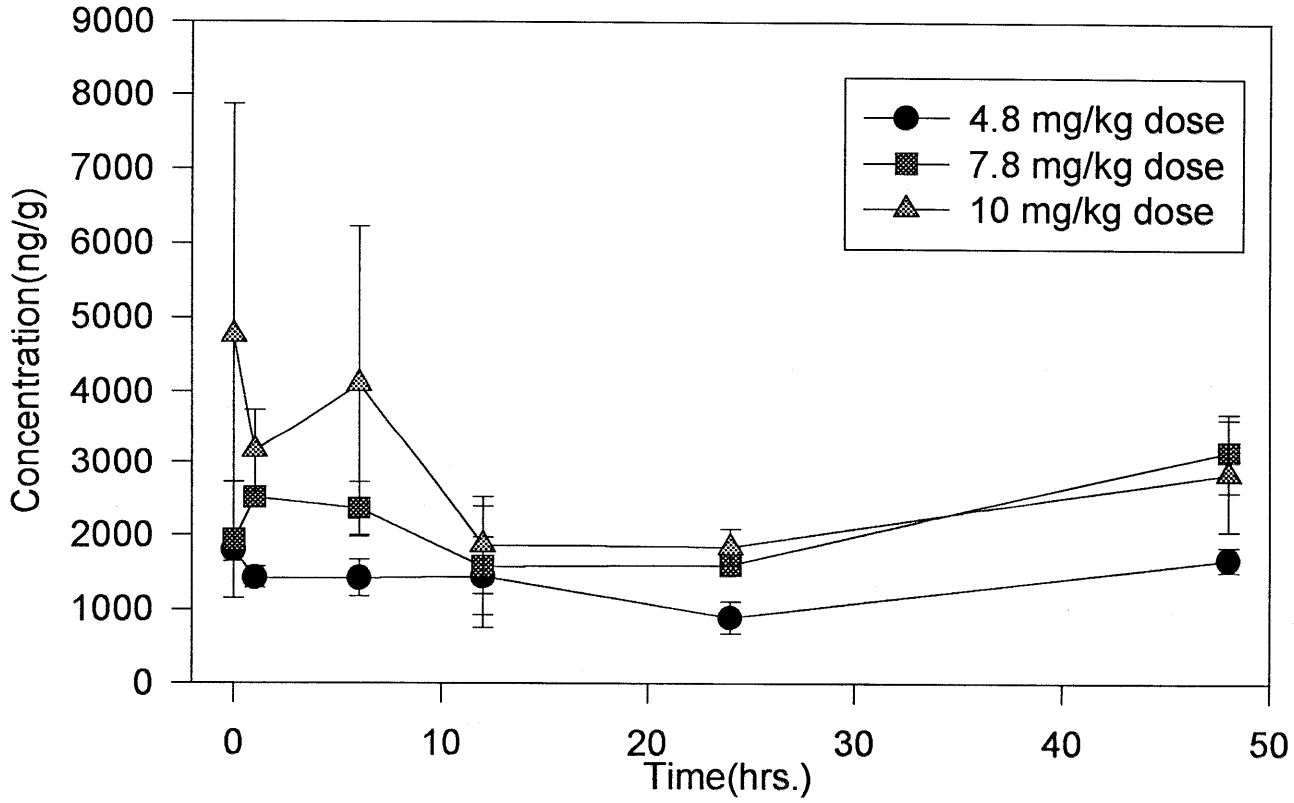
ALPRAZOLAM CONCENTRATION IN LIVER (NG/G)



ALPRAZOLAM CONCENTRATION IN LIVER (NG/G)Regression Order:2

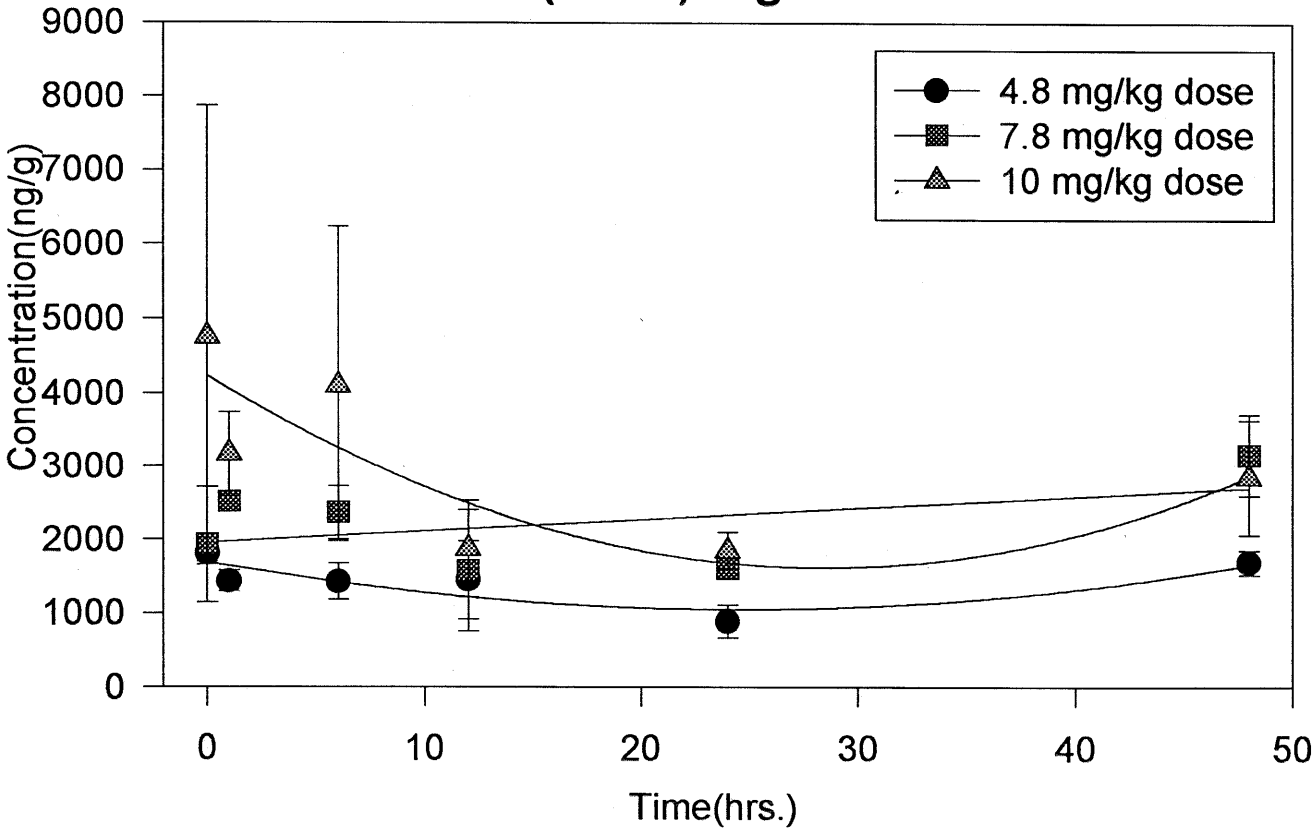
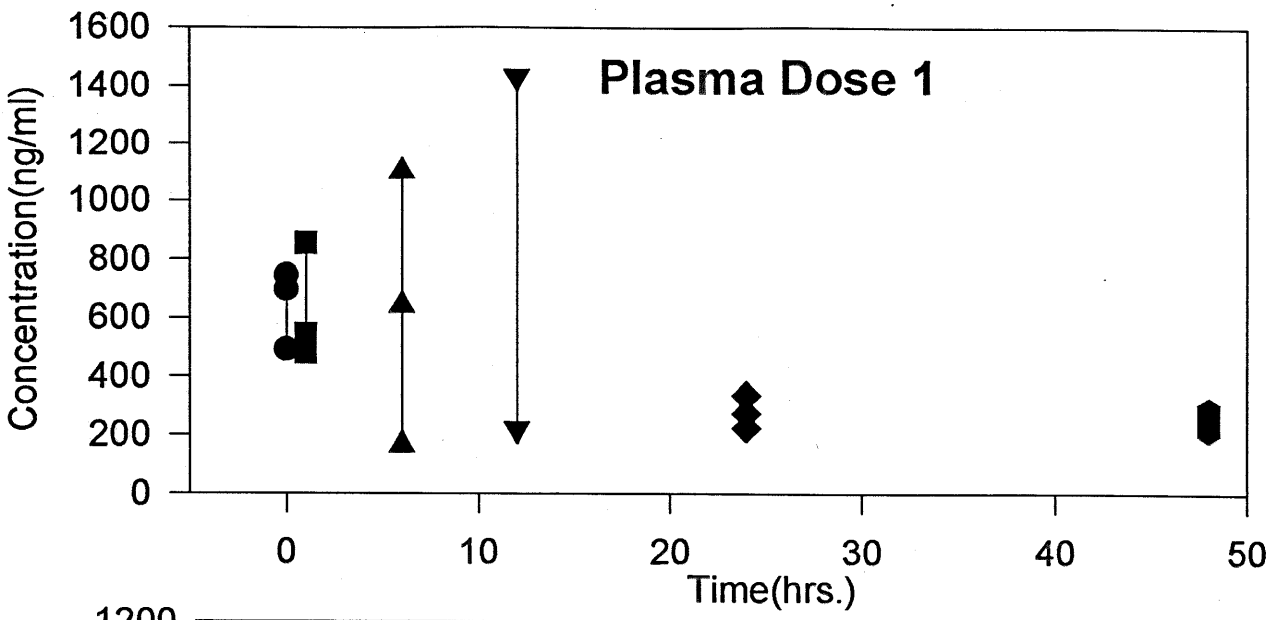
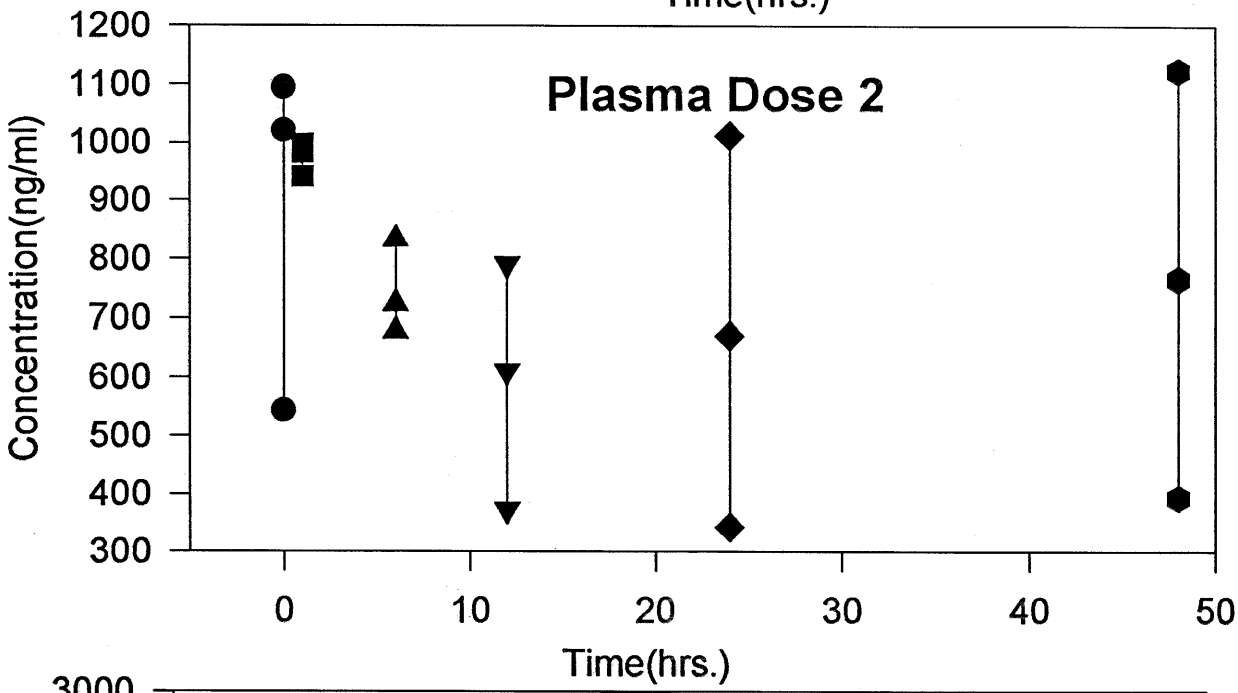


FIGURE 35. Plasma data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three alprazolam dosages (dose 1 = 4.8 mg/kg, dose 2 = 7.8 mg/kg, and dose 3 = 10 mg/kg)

(figure on next page)


Plasma Dose 1
Concentration(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time(hrs.)


Plasma Dose 2
Concentration(ng/ml)
1200
1100
1000
900
800
700
600
500
400
300
0
10
20
30
40
50
Time(hrs.)

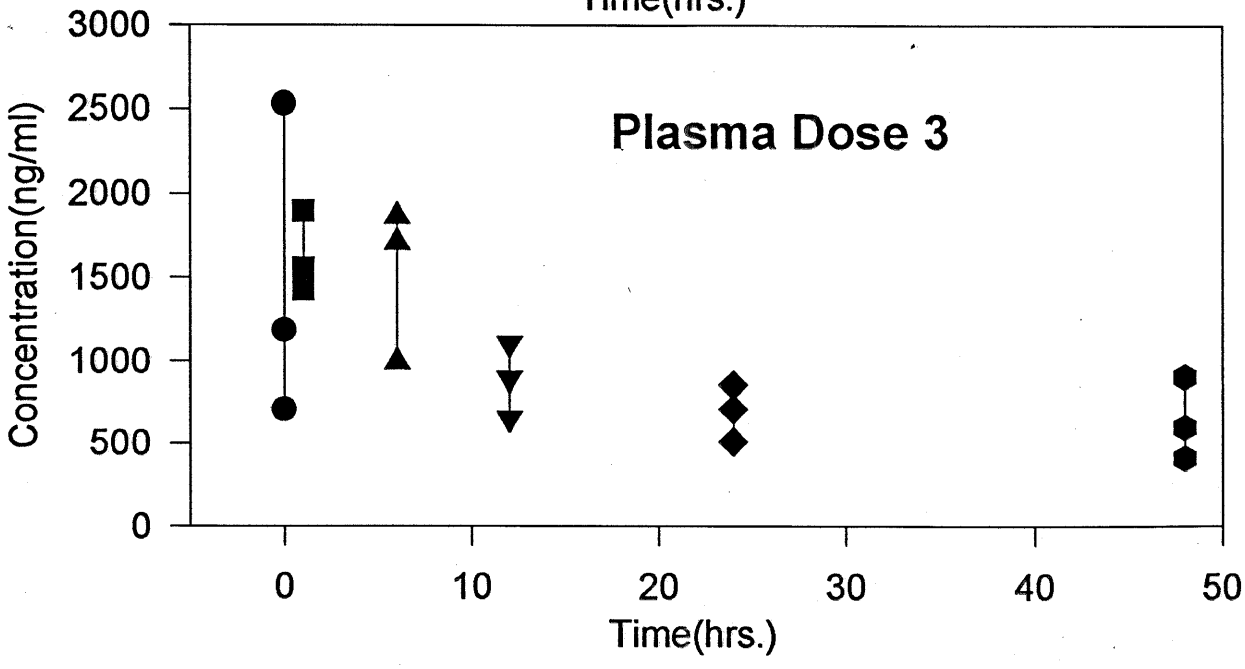
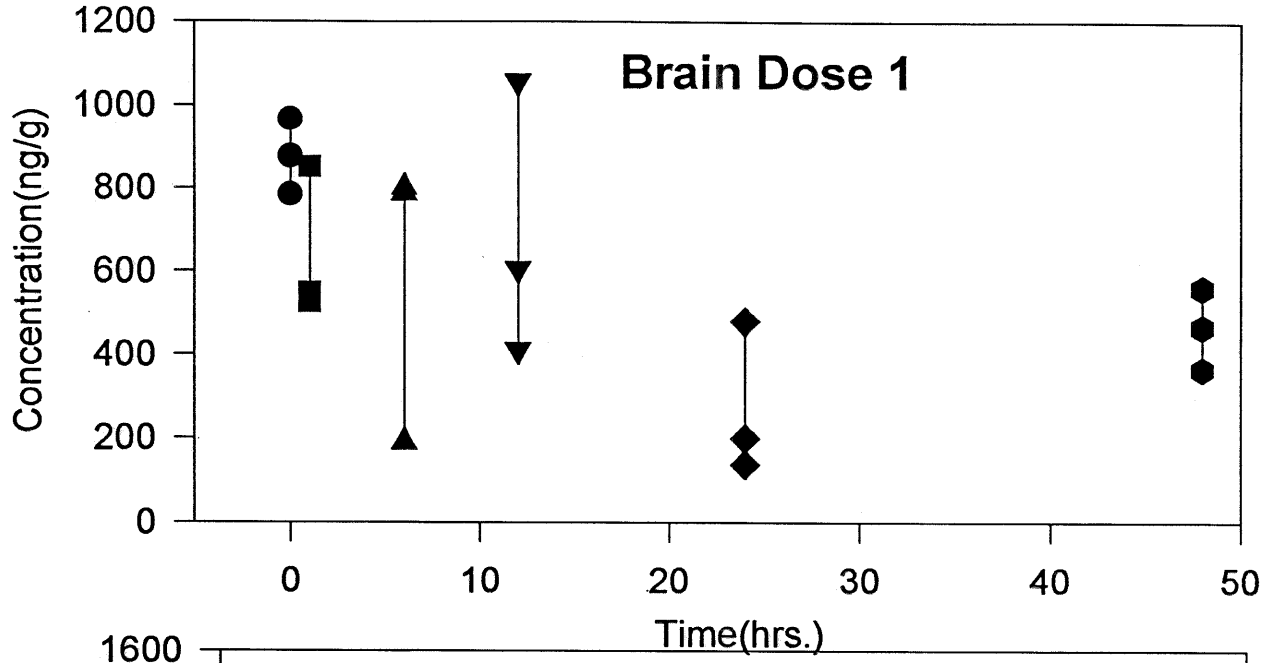
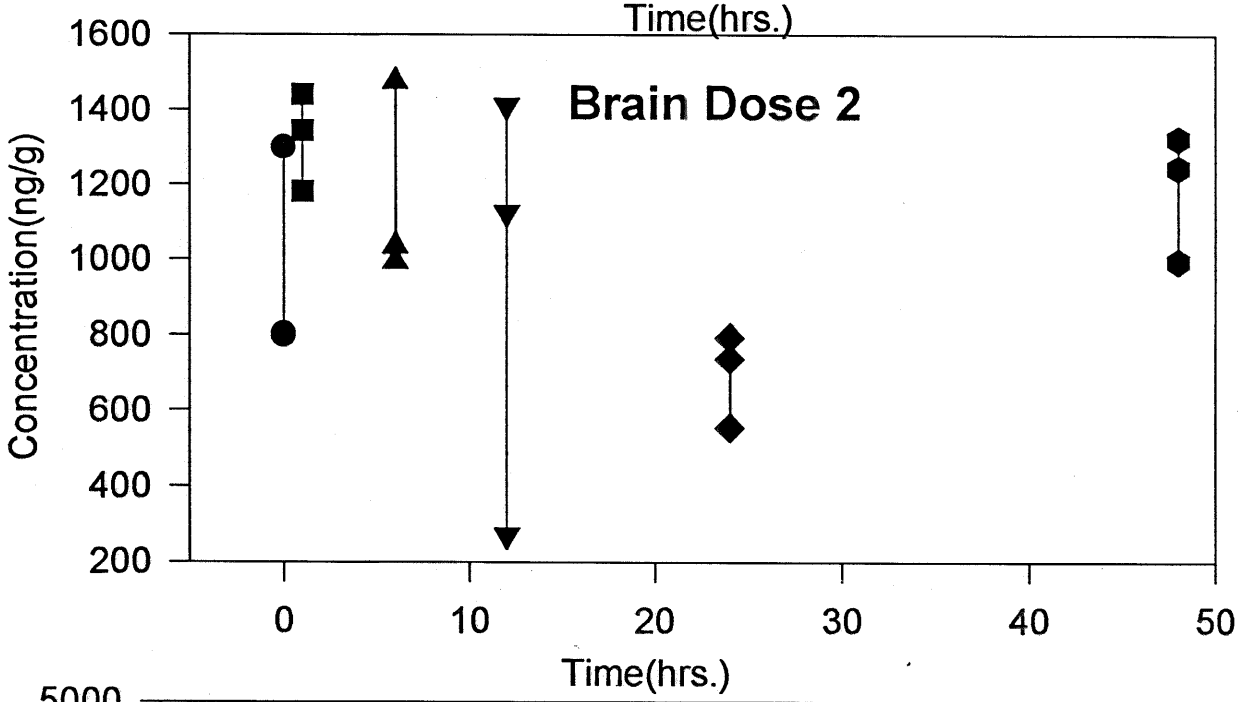

Plasma Dose 3
Concentration(ng/ml)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 36. Brain data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three alprazolam dosages (dose 1 = 4.8 mg/kg, dose 2 = 7.8 mg/kg, and dose 3 = 10 mg/kg)

(figure on next page)


Brain Dose 1
Concentration(ng/g)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time(hrs.)


Brain Dose 2
Concentration(ng/g)
1600
1400
1200
1000
800
600
400
200
0
10
20
30
40
50
Time(hrs.)

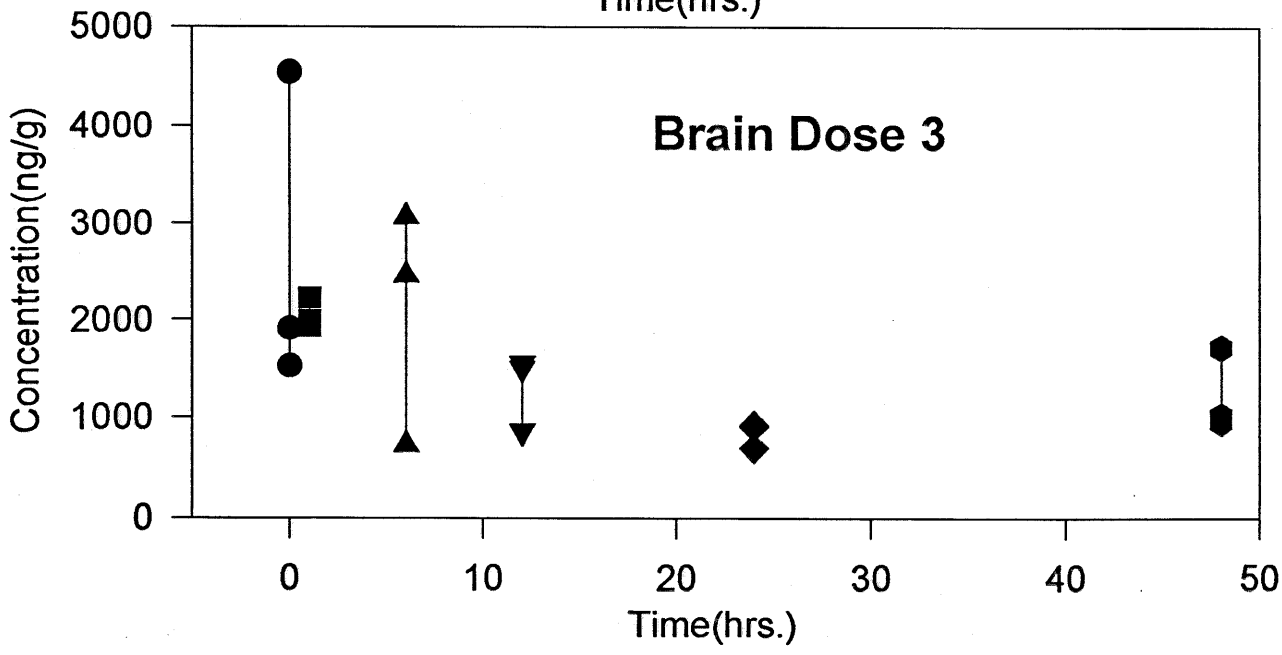
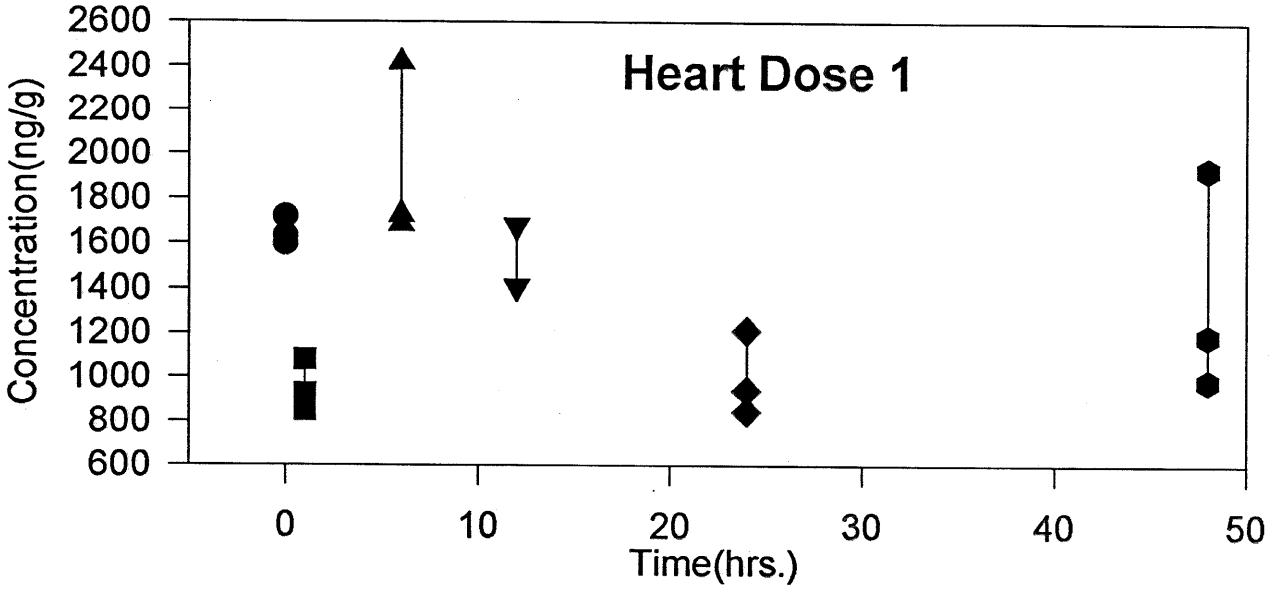
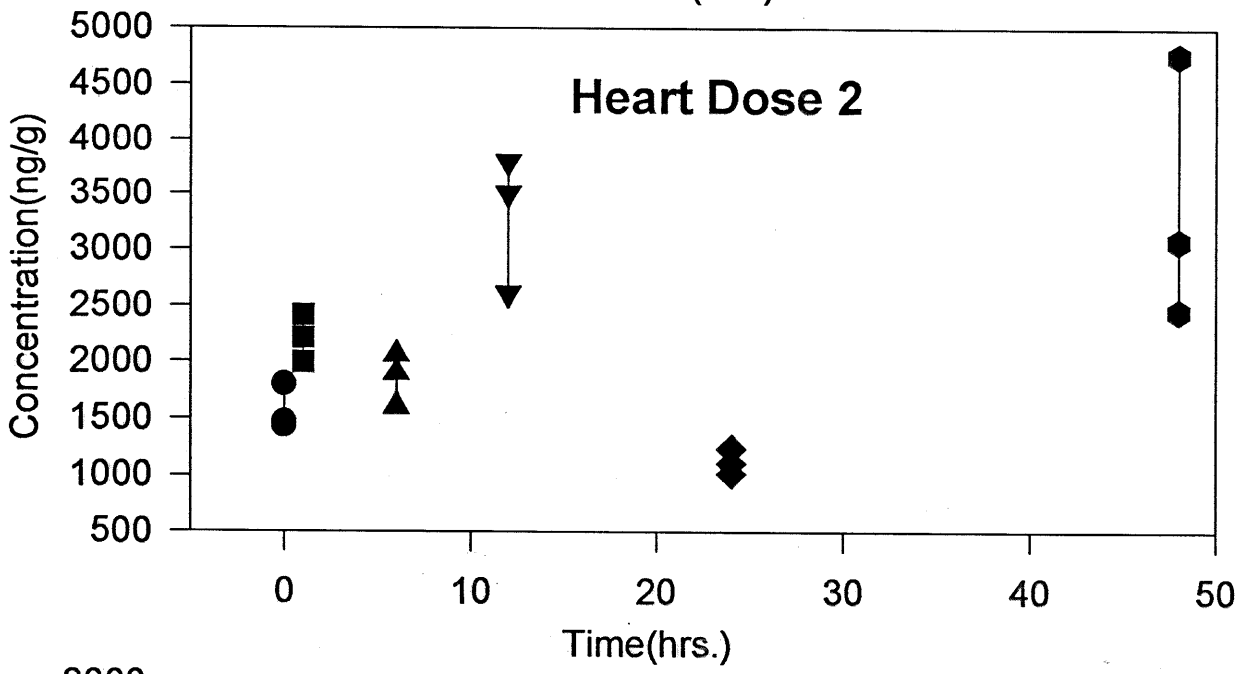
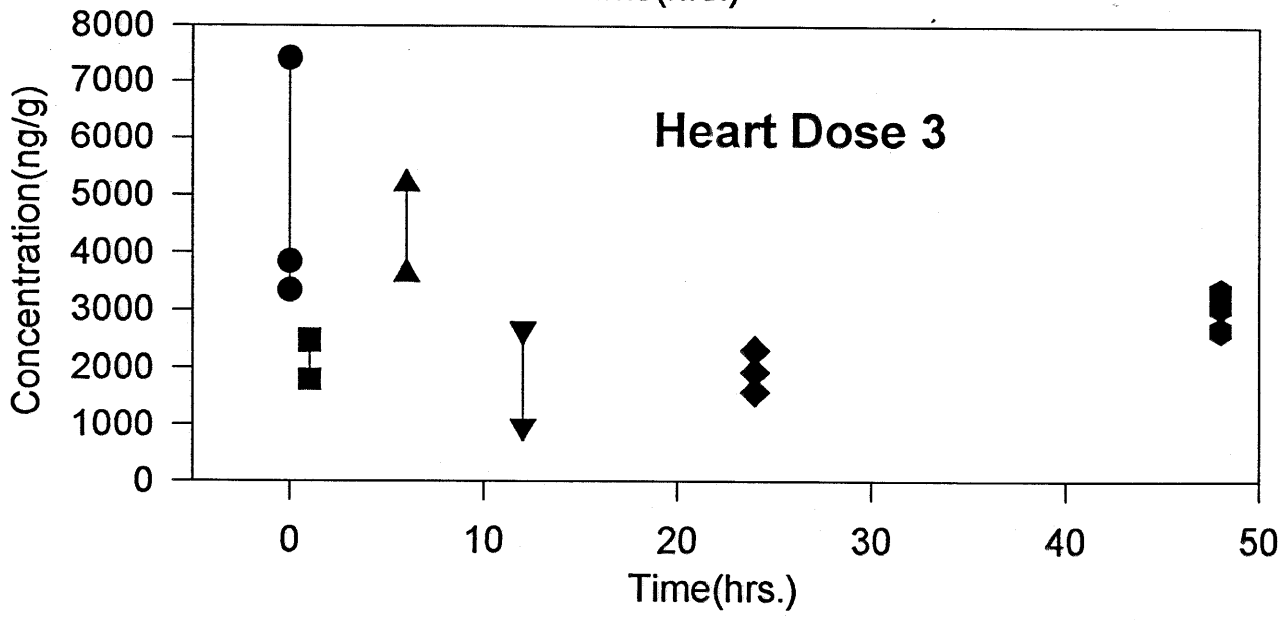

Brain Dose 3
Concentration(ng/g)
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)

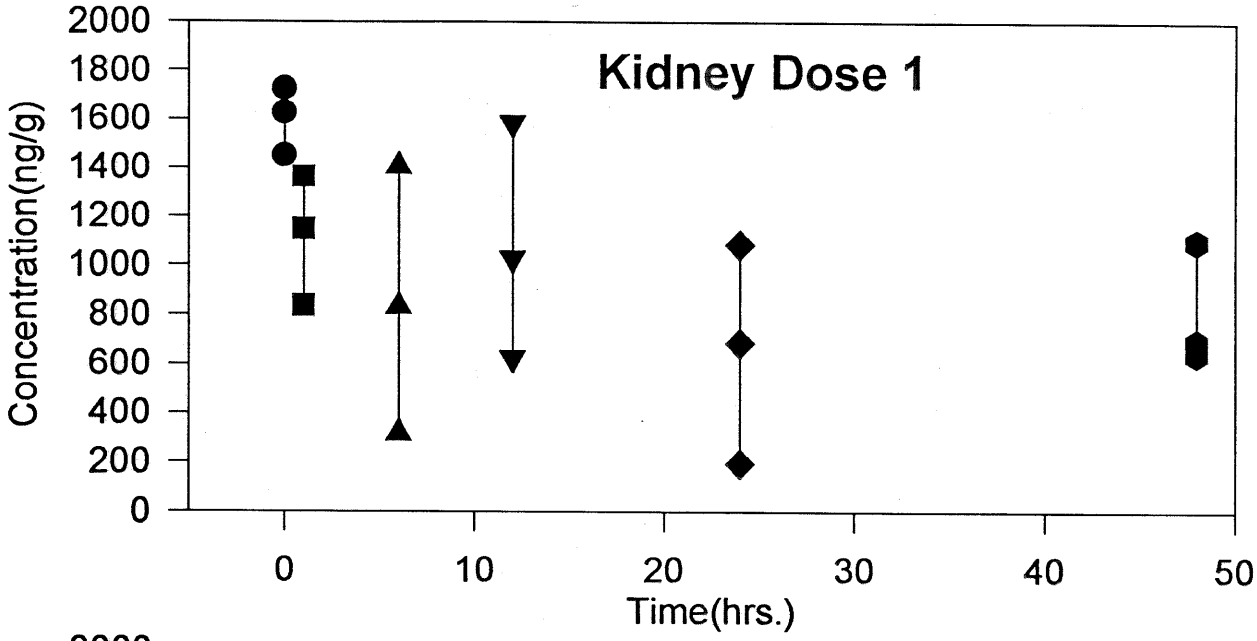
FIGURE 37. Heart data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three alprazolam dosages (dose 1 = 4.8 mg/kg, dose 2 = 7.8 mg/kg, and dose 3 = 10 mg/kg)

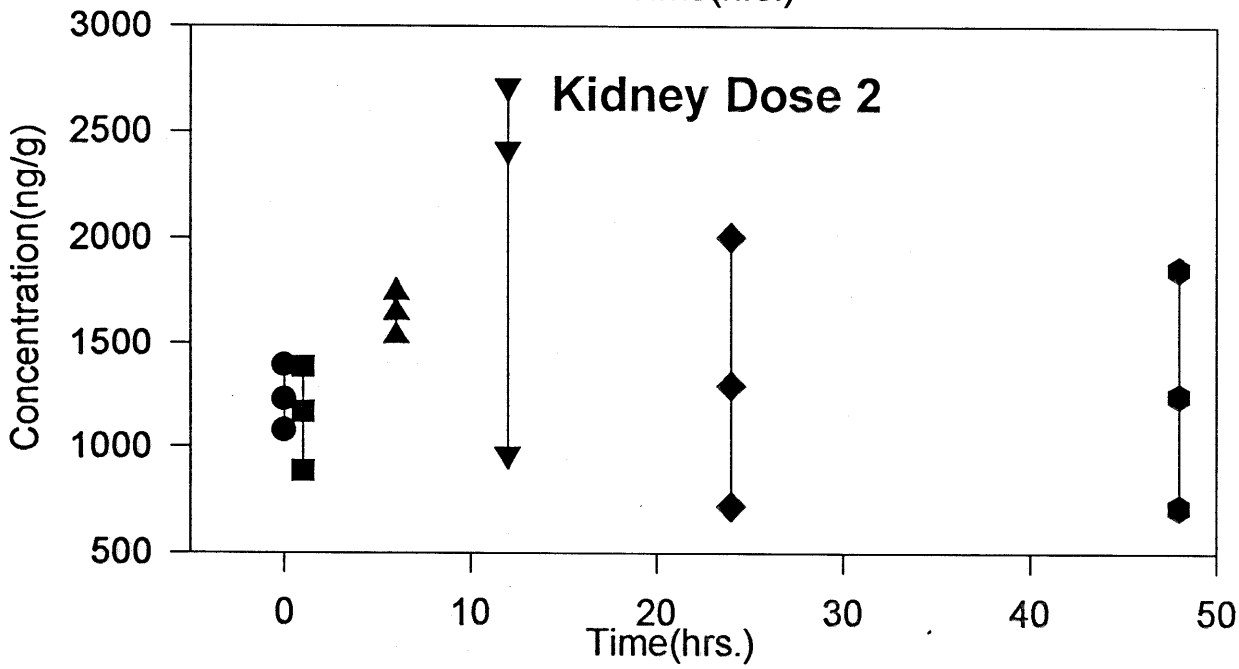
(figure on next page)


Heart Dose 1
Concentration(ng/g)
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
0
10
20
30
40
50
Time(hrs.)


Heart Dose 2
Concentration(ng/g)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10
20
30
40
50
Time(hrs.)


Heart Dose 3
Concentration(ng/g)
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)


Kidney Dose 1
Concentration(ng/g)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time(hrs.)


Kidney Dose 2
Concentration(ng/g)
3000
2500
2000
1500
1000
500
0
10
20
30
40
50
Time(hrs.)

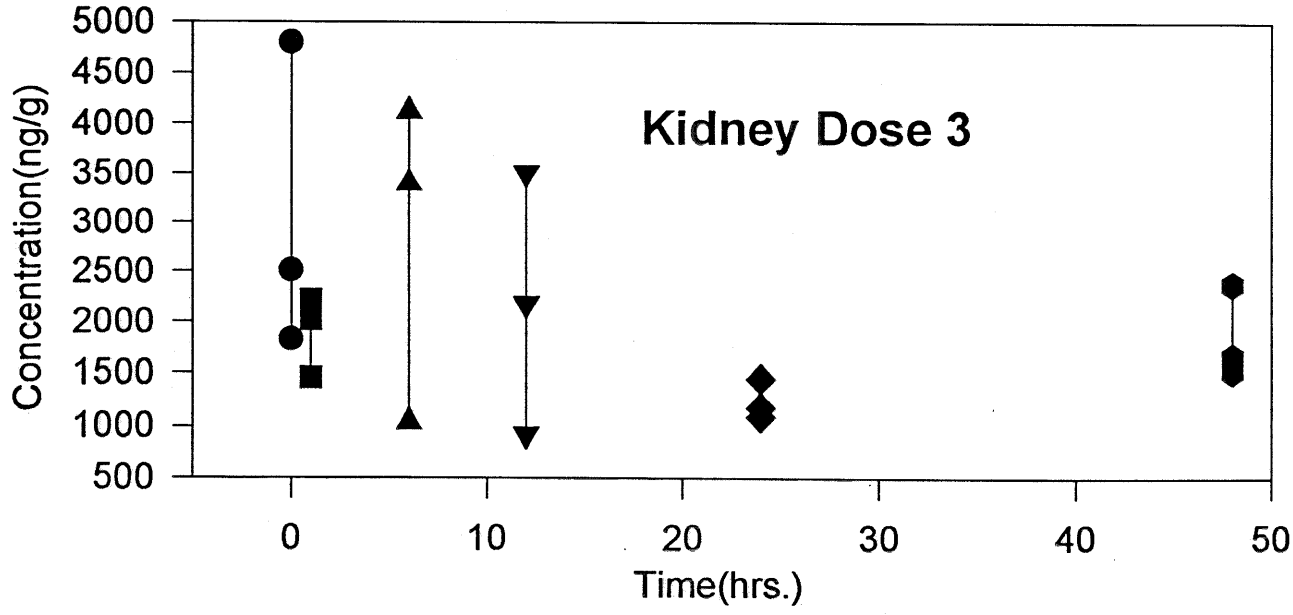
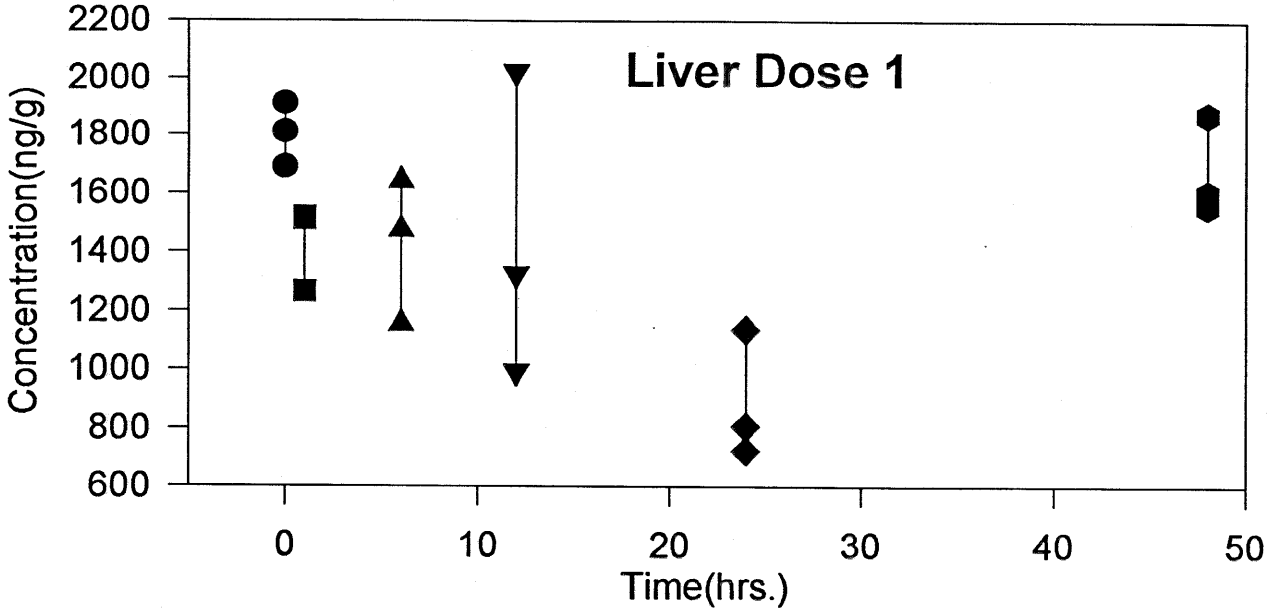
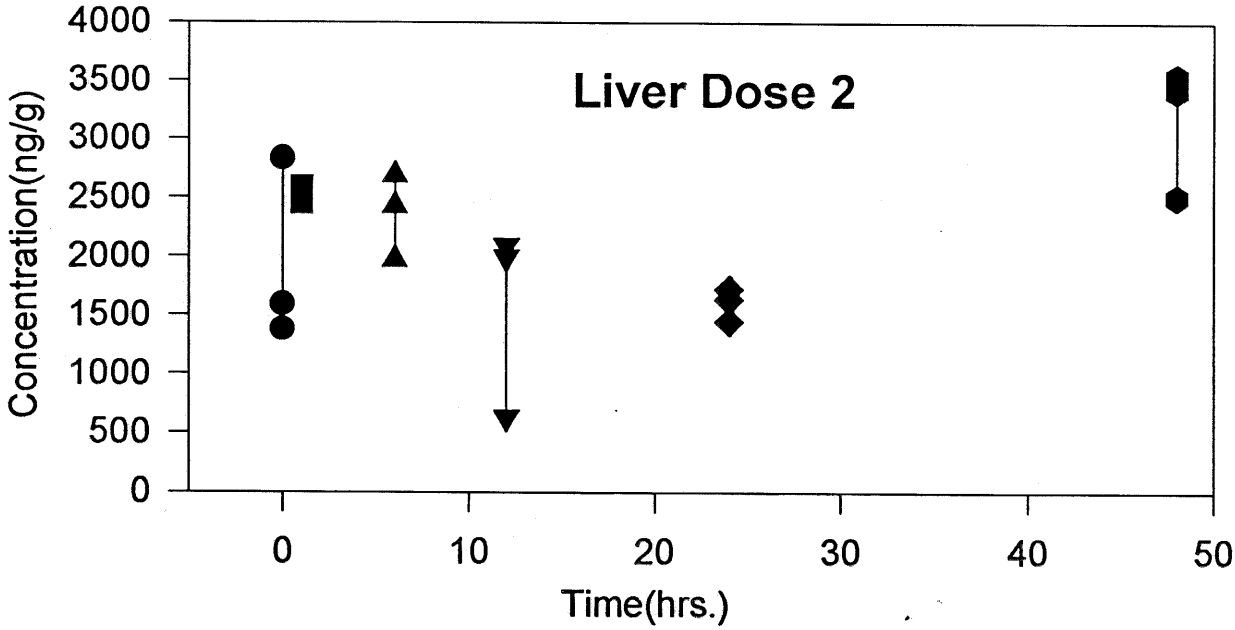
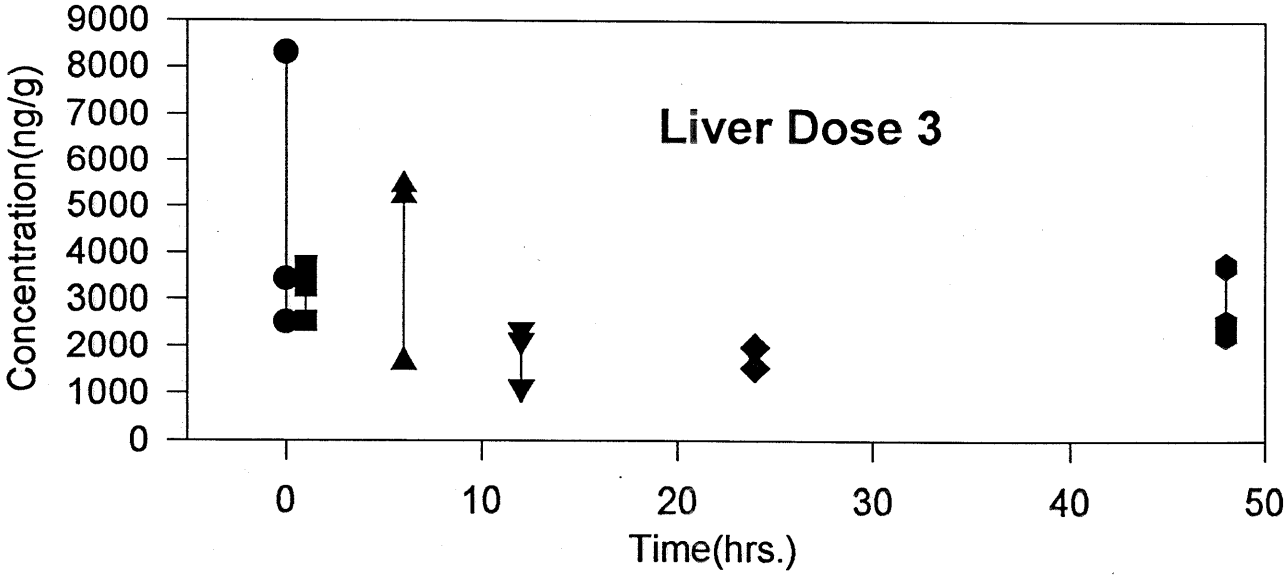

Kidney Dose 3
Concentration(ng/g)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10
20
30
40
50
Time(hrs.)

FIGURE 39. Liver data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three alprazolam dosages (dose 1 = 4.8 mg/kg, dose 2 = 7.8 mg/kg, and dose 3 = 10 mg/kg)

(figure on next page)


Liver Dose 1
Concentration(ng/g)
2200
2000
1800
1600
1400
1200
1000
800
600
0
10
20
30
40
50
Time(hrs.)


Liver Dose 2
Concentration(ng/g)
4000
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)


Liver Dose 3
Concentration(ng/g)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)

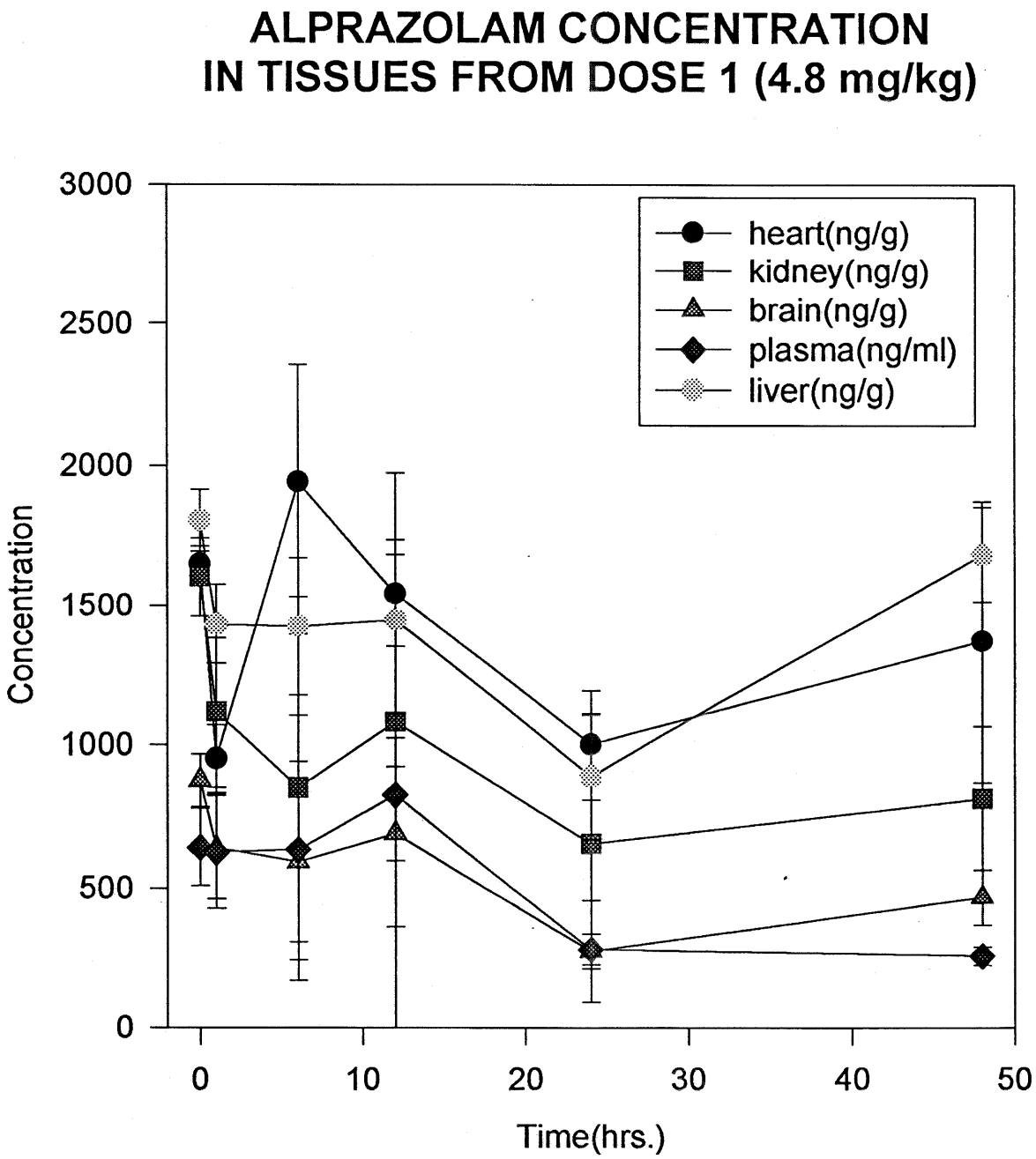


FIGURE 40. Point-to-point analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 4.8 mg/kg dose

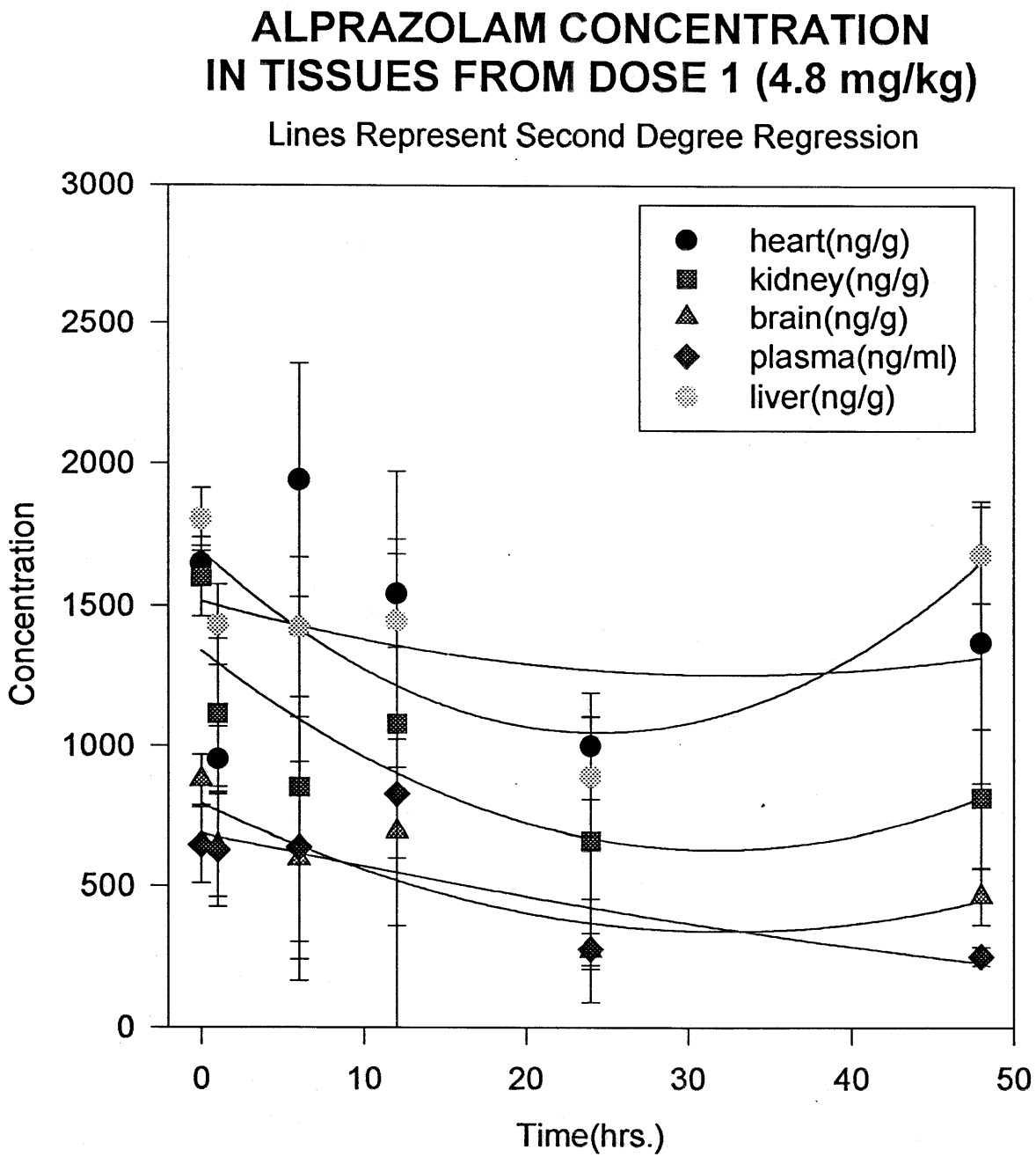


FIGURE 41. Second-order regression analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 4.8 mg/kg dose

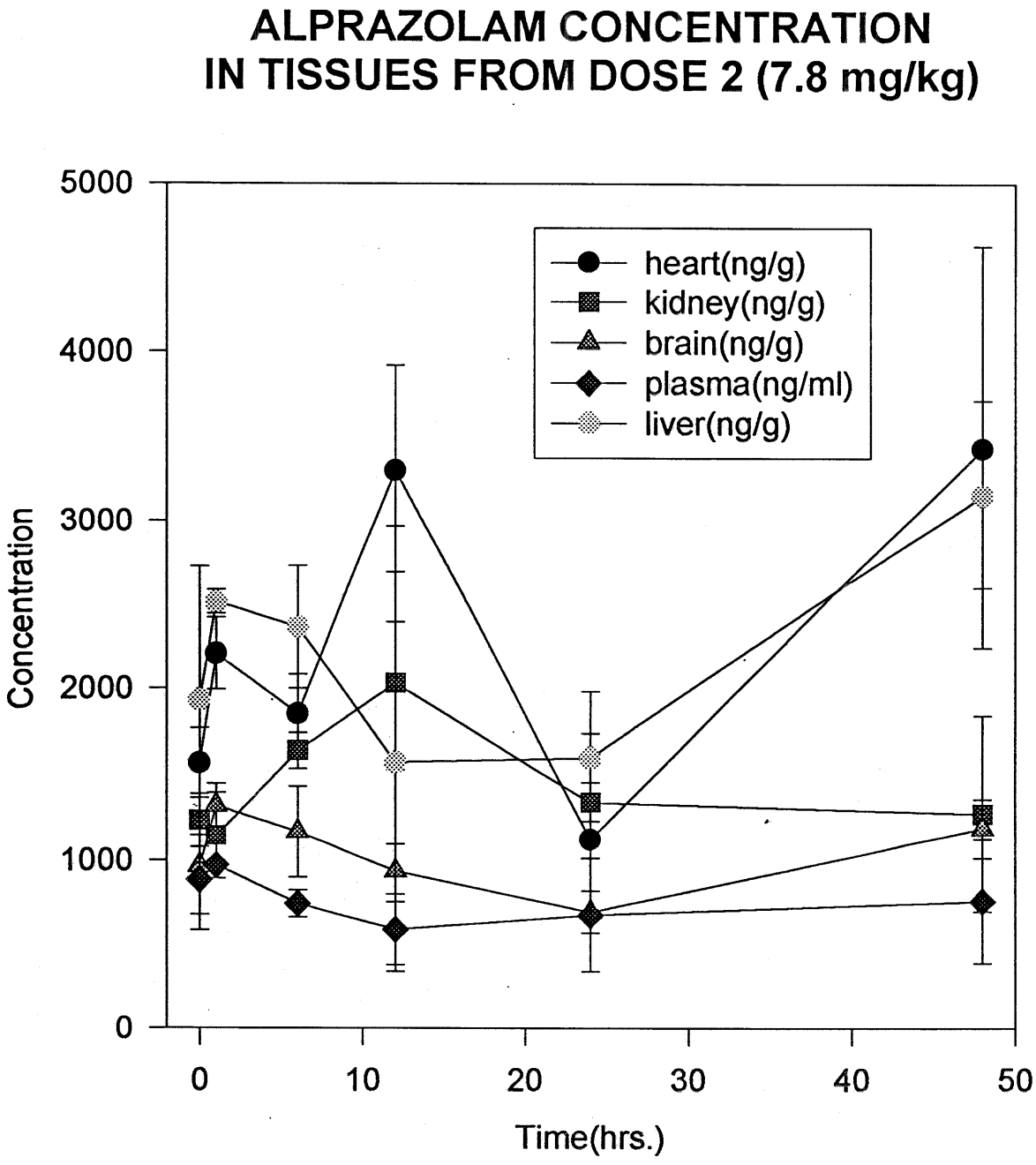


FIGURE 42. Point-to-point analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 7.8 mg/kg dose

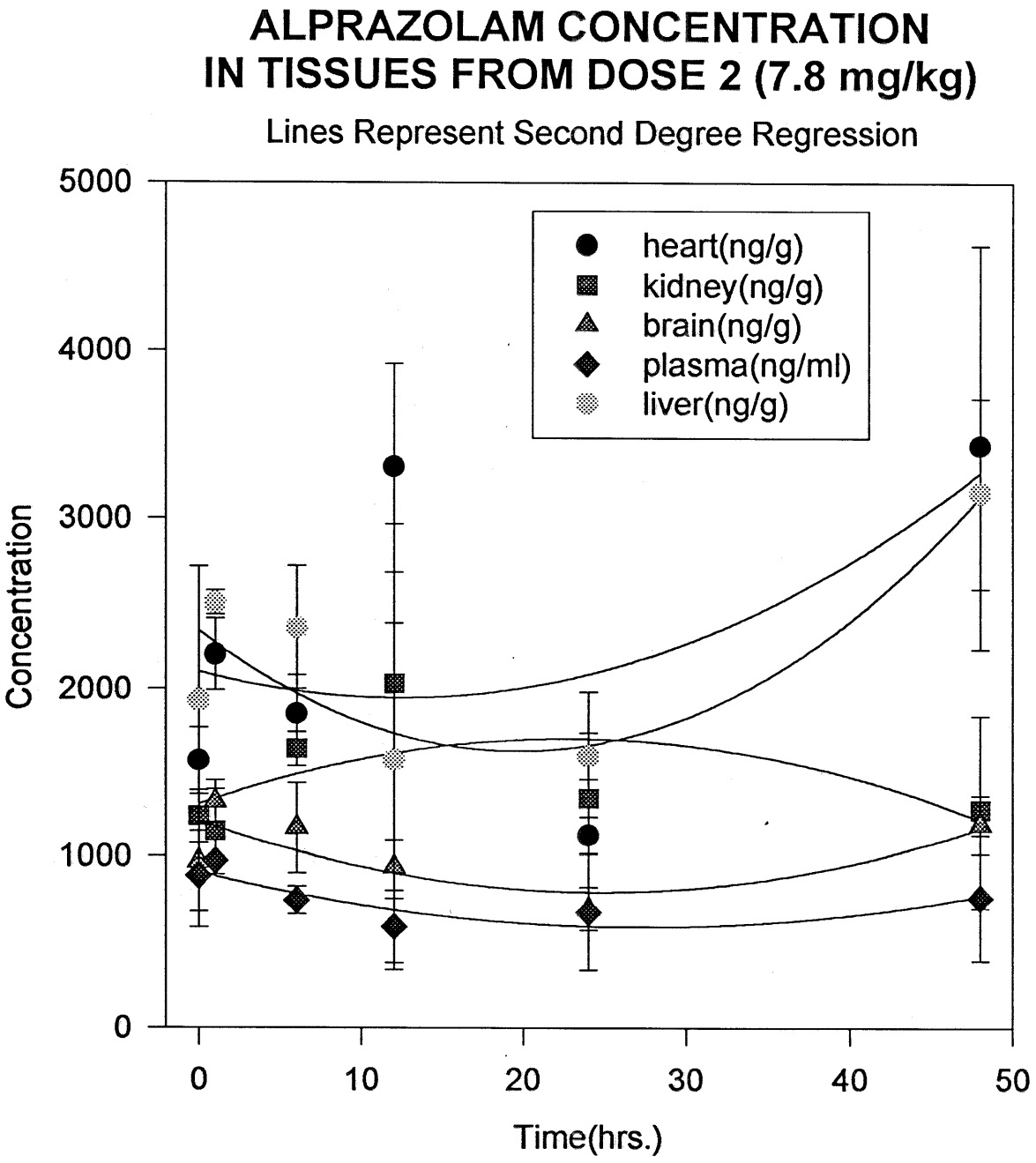


FIGURE 43. Second-order regression analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 7.8 mg/kg dose

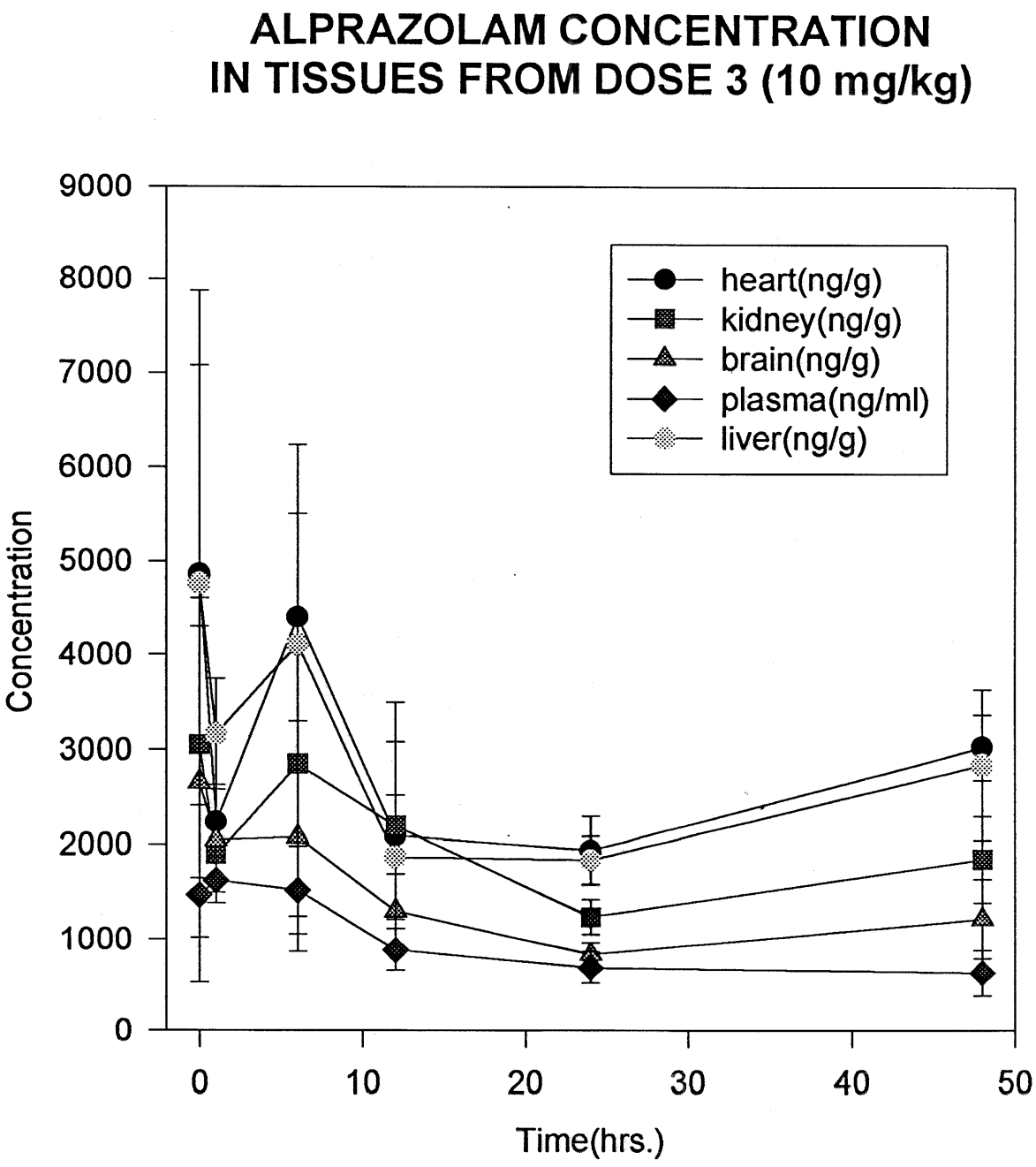


FIGURE 44. Point-to-point analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 10 mg/kg dose

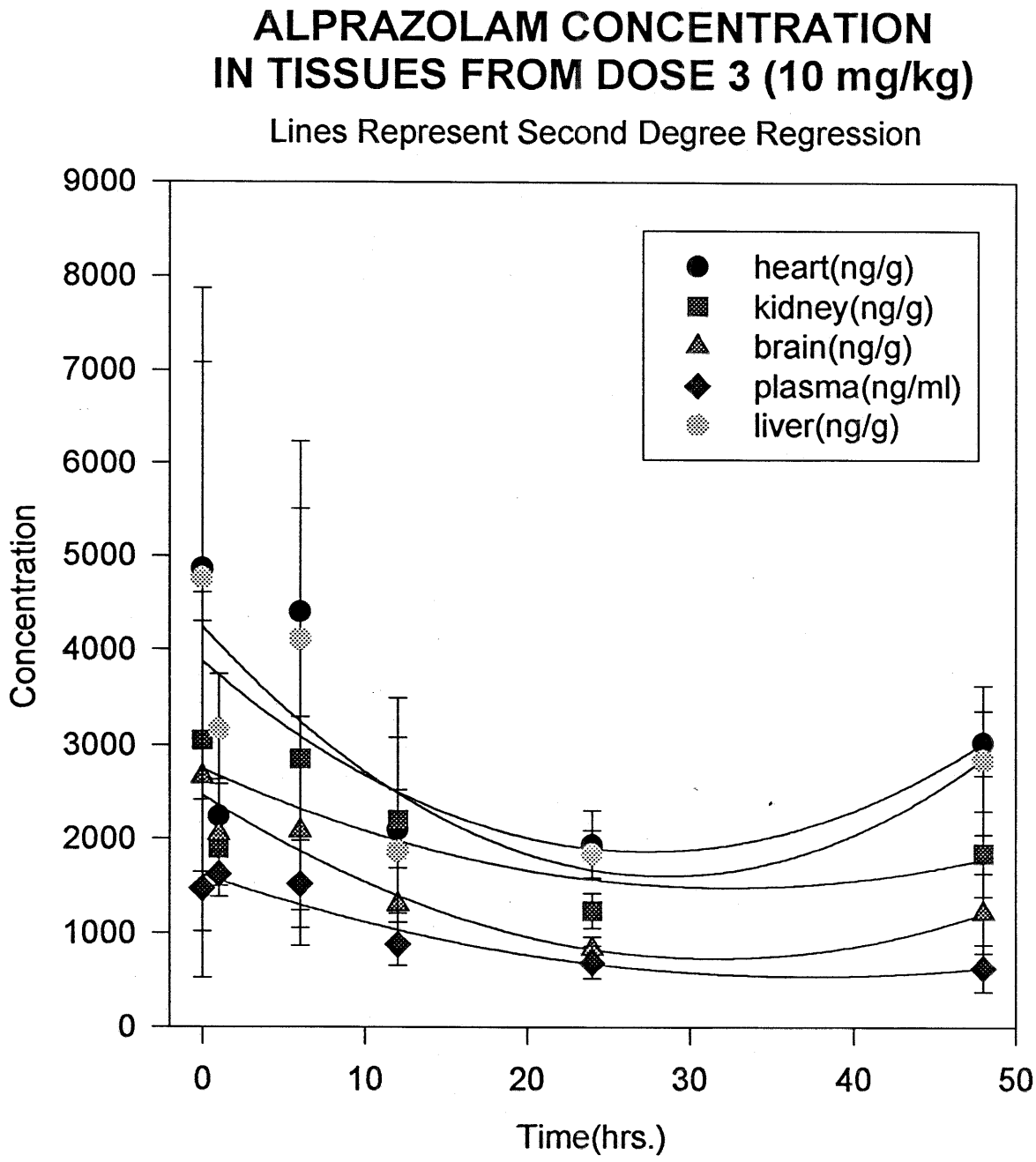
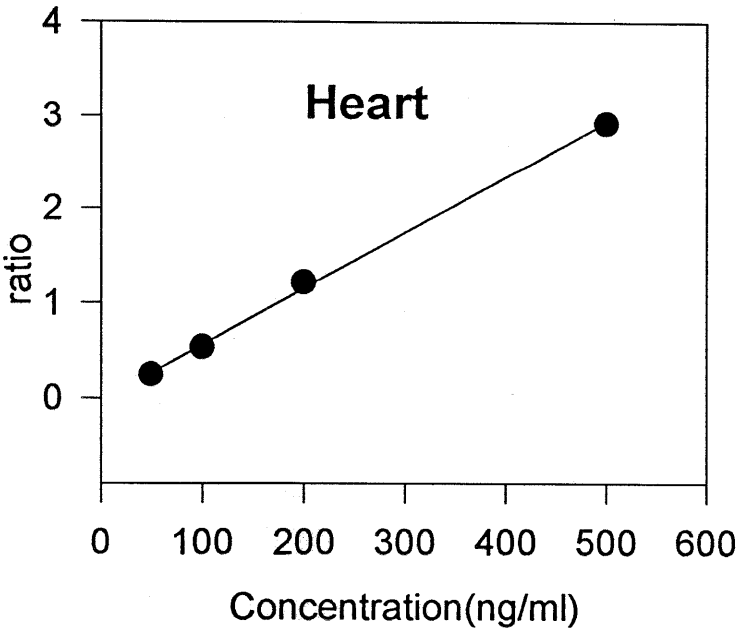
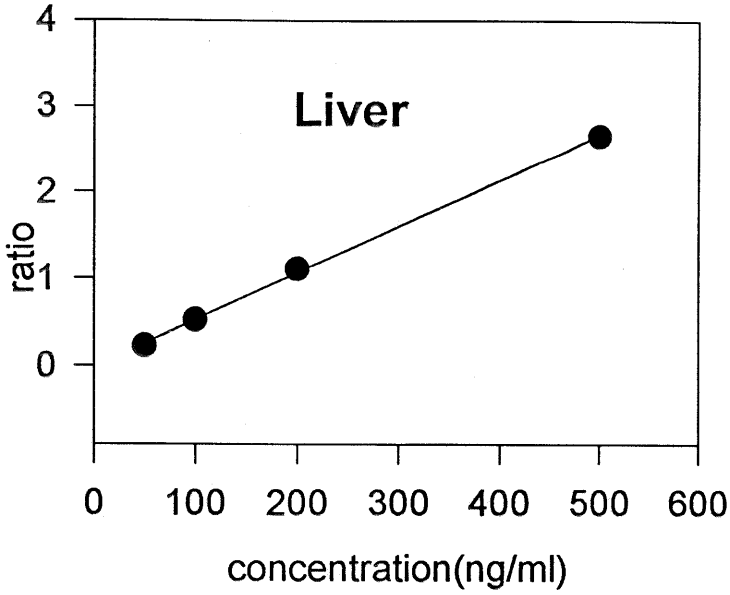
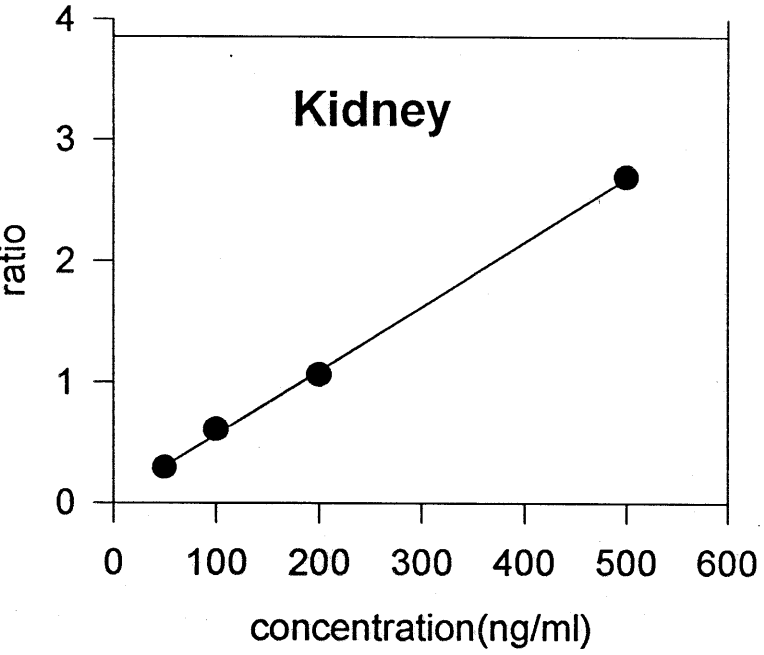
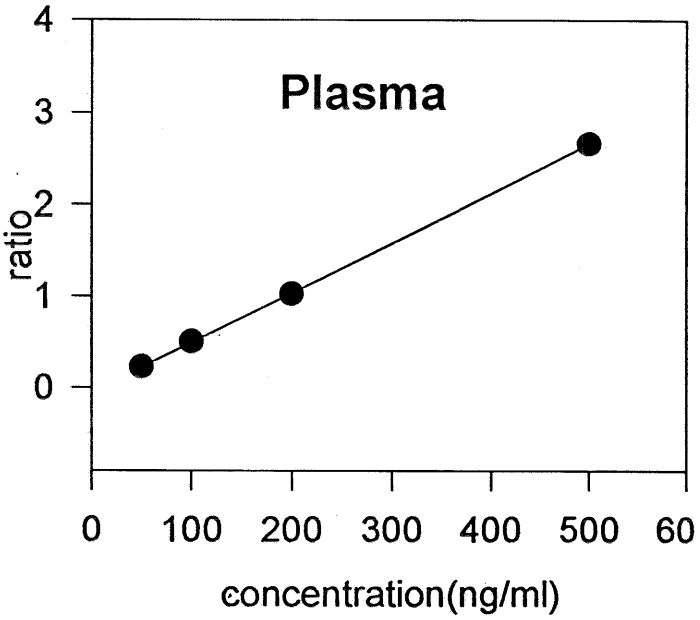


FIGURE 45. Second-order regression analysis of alprazolam concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 10 mg/kg dose









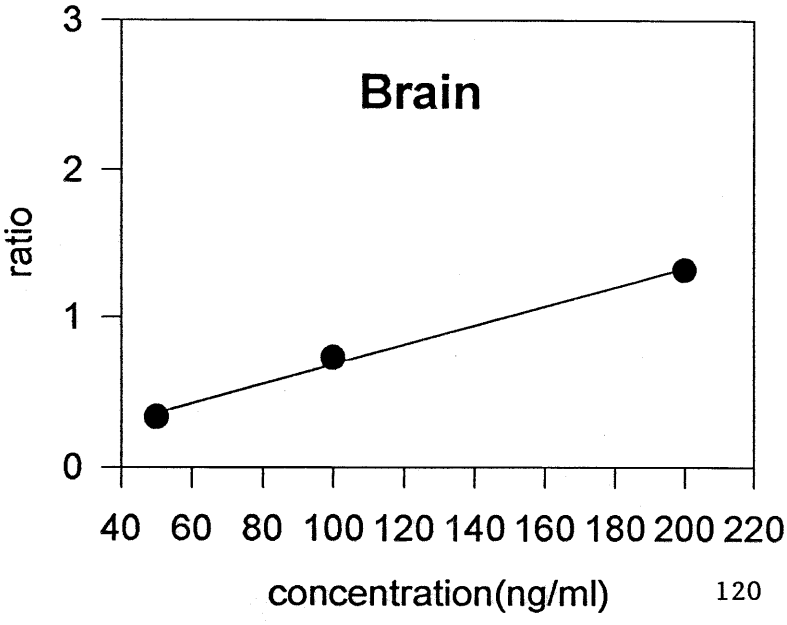
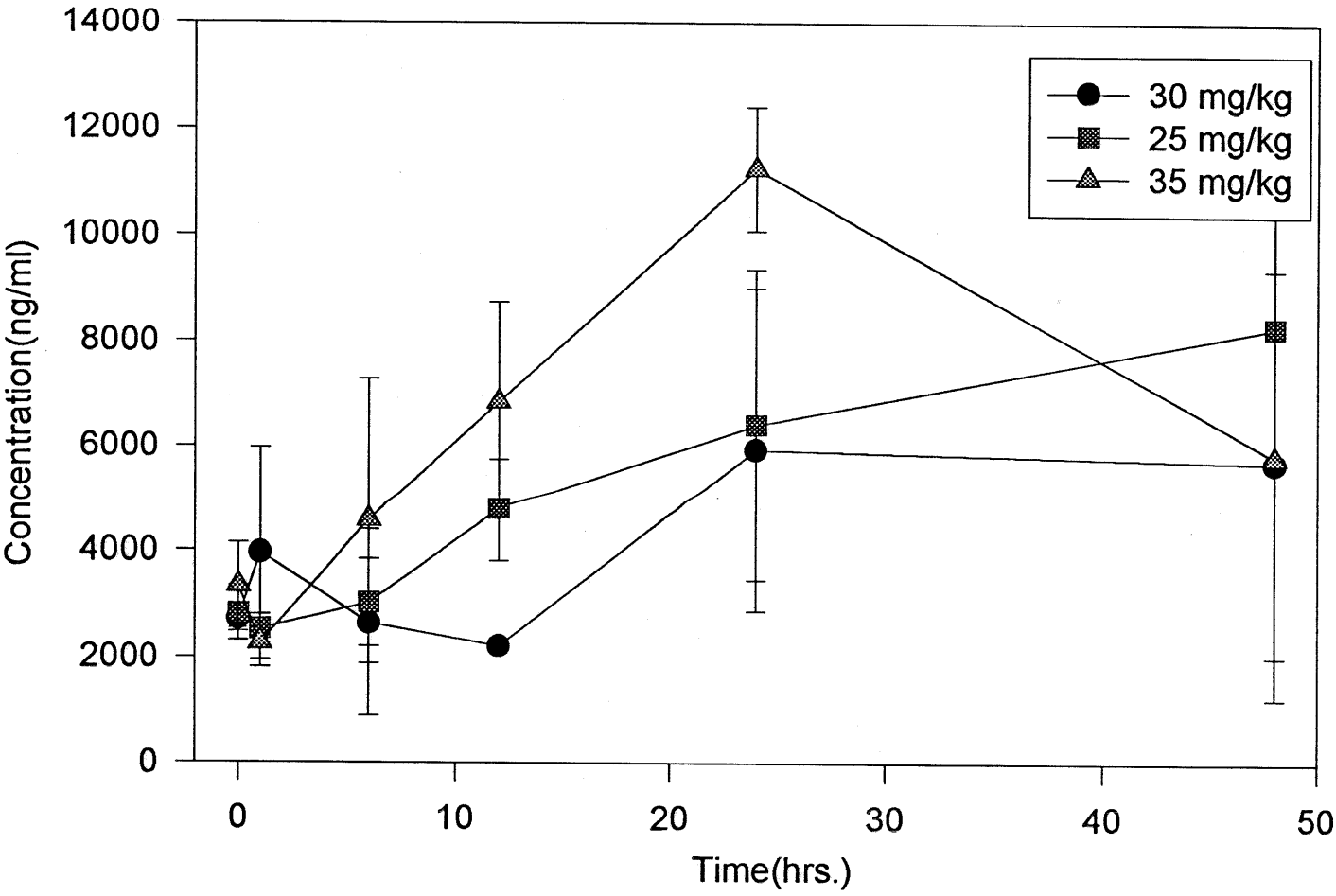


FIGURE 46. Graphs of standard curve(concentration vs. the ratio of drug/internal standard) for atenolol in heart, kidney, brain, liver, and plasma

FIGURE 47. Atenolol concentration in plasma (ng/ml, mean $\pm$ S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

ATENOLOL CONCENTRATION IN PLASMA(NG/ML)



ATENOLOL CONCENTRATION IN PLASMA(NG/ML)Regression Order:2

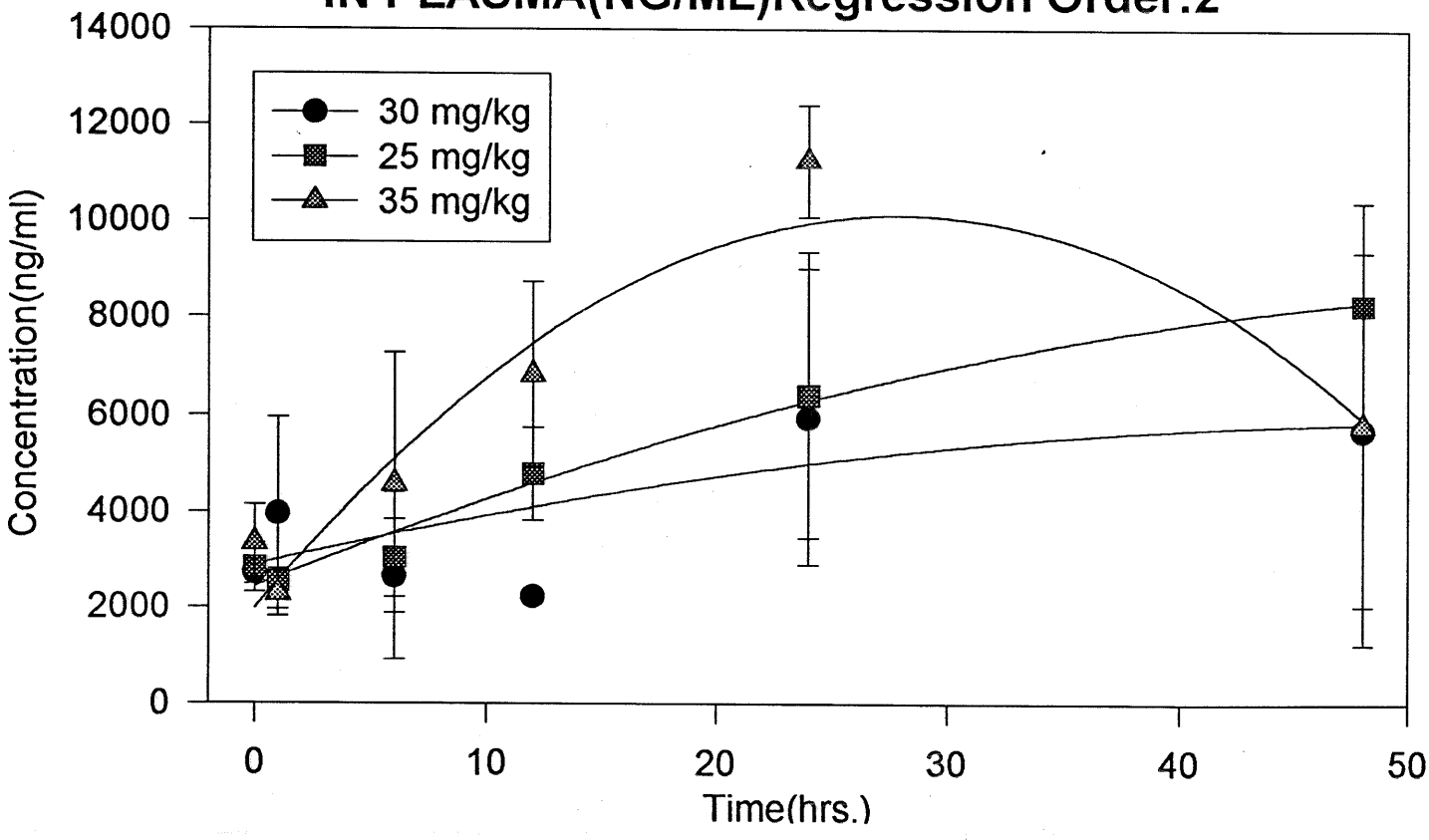
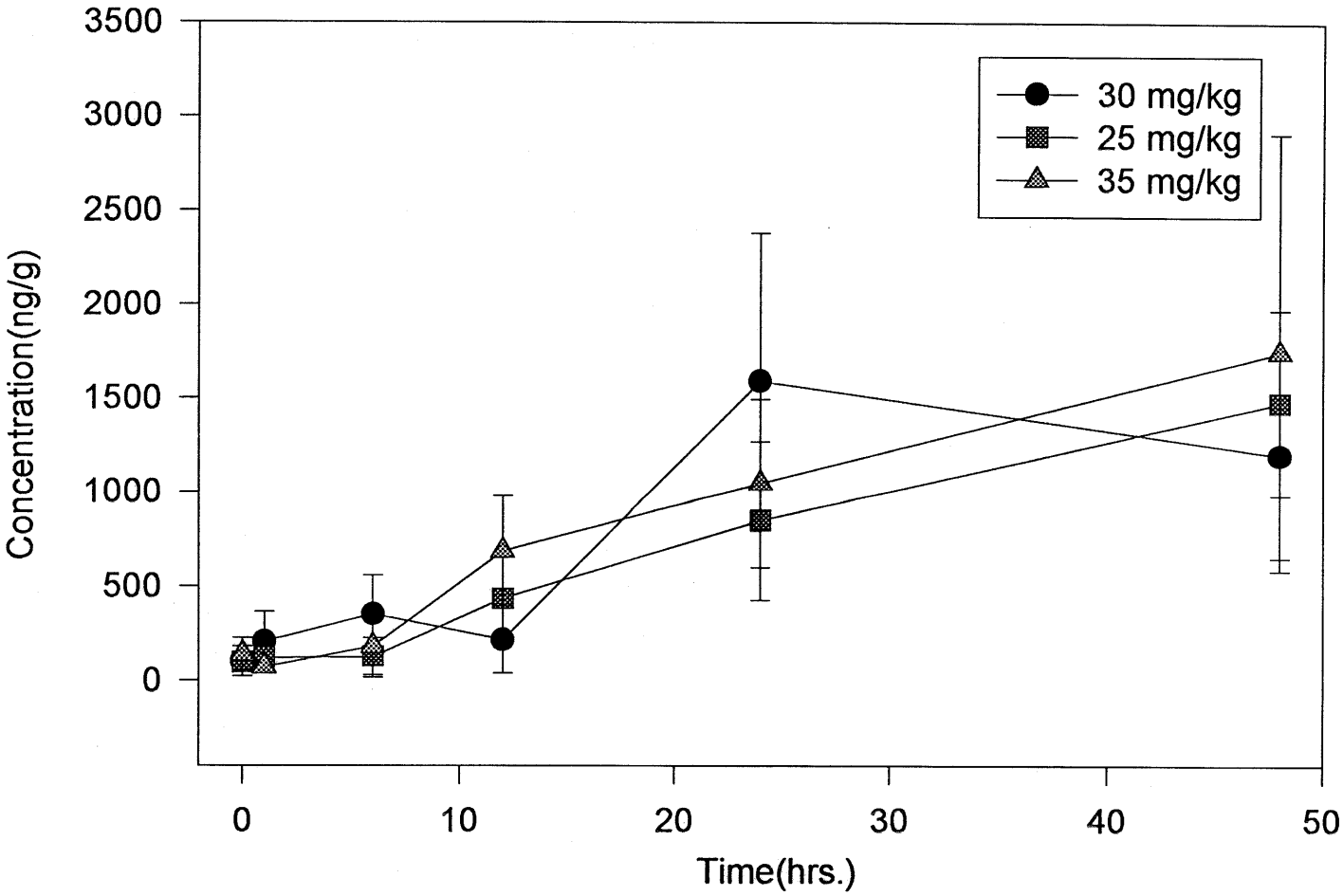


FIGURE 48. Atenolol concentration in brain (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

ATENOLOL CONCENTRATION IN BRAIN(NG/G)



ATENOLOL CONCENTRATION IN BRAIN(NG/G)Regression Order:2

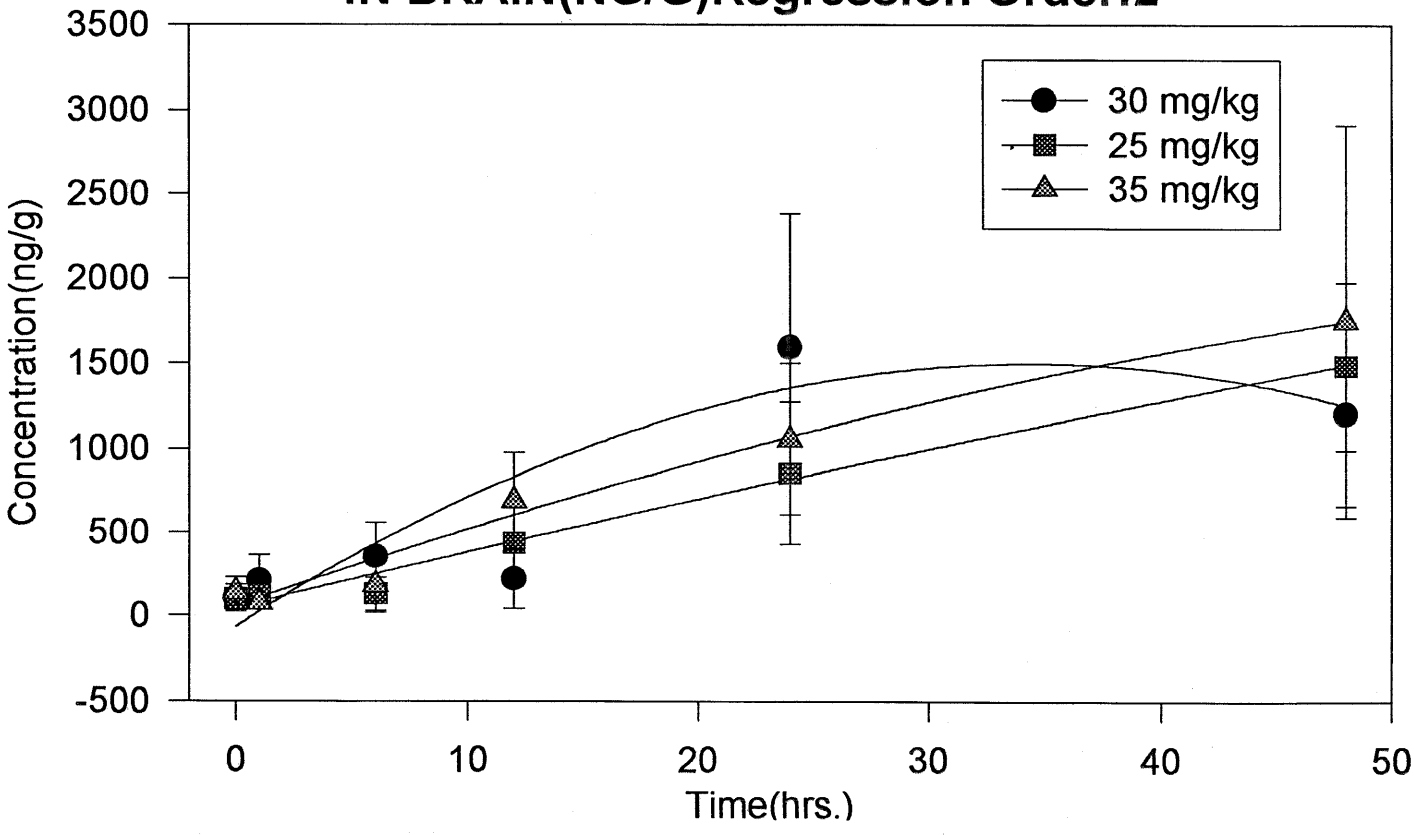
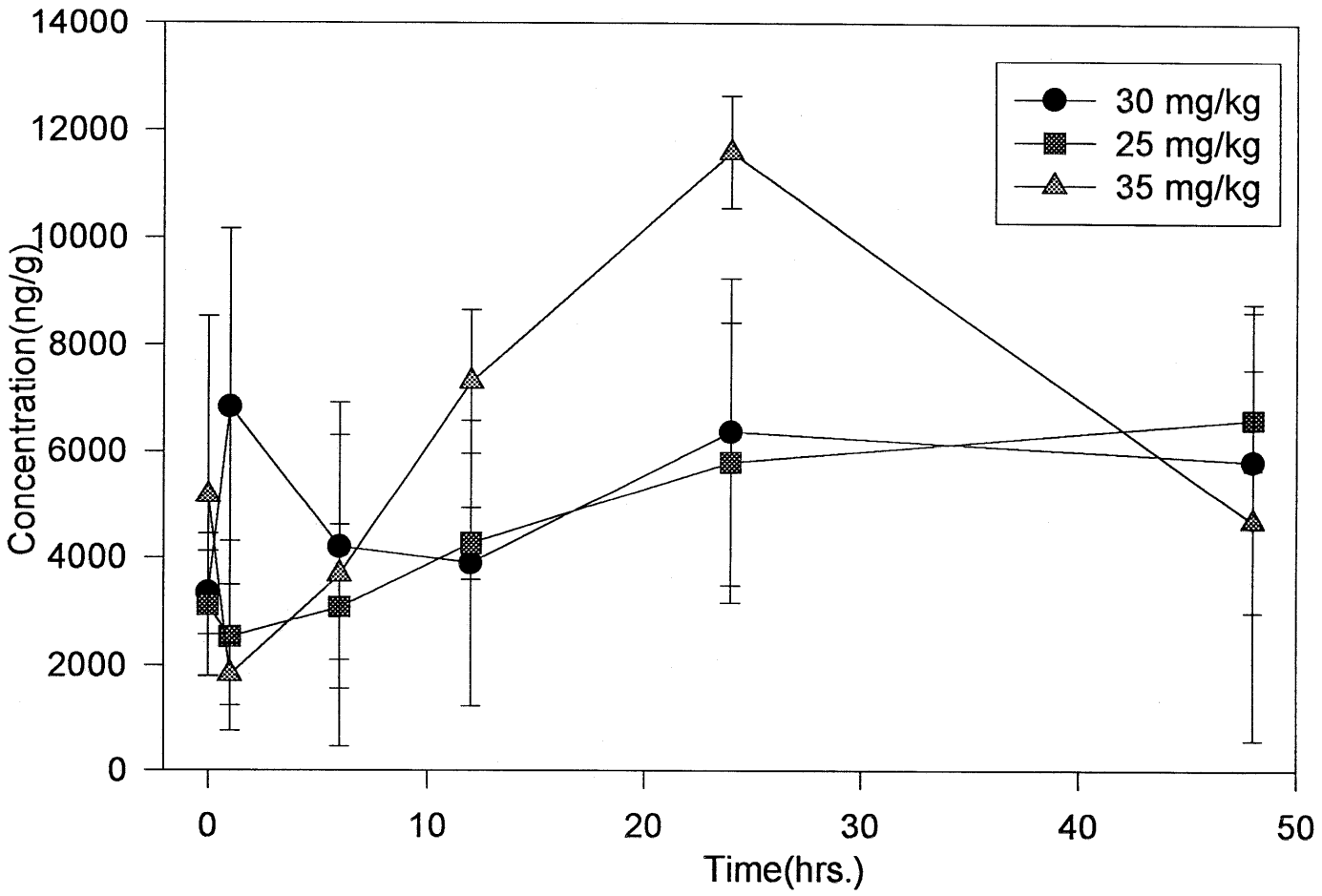


FIGURE 49. Atenolol concentration in heart (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

ATENOLOL CONCENTRATION IN HEART(NG/G)



ATENOLOL CONCENTRATION IN HEART(NG/G)Regression Order:2

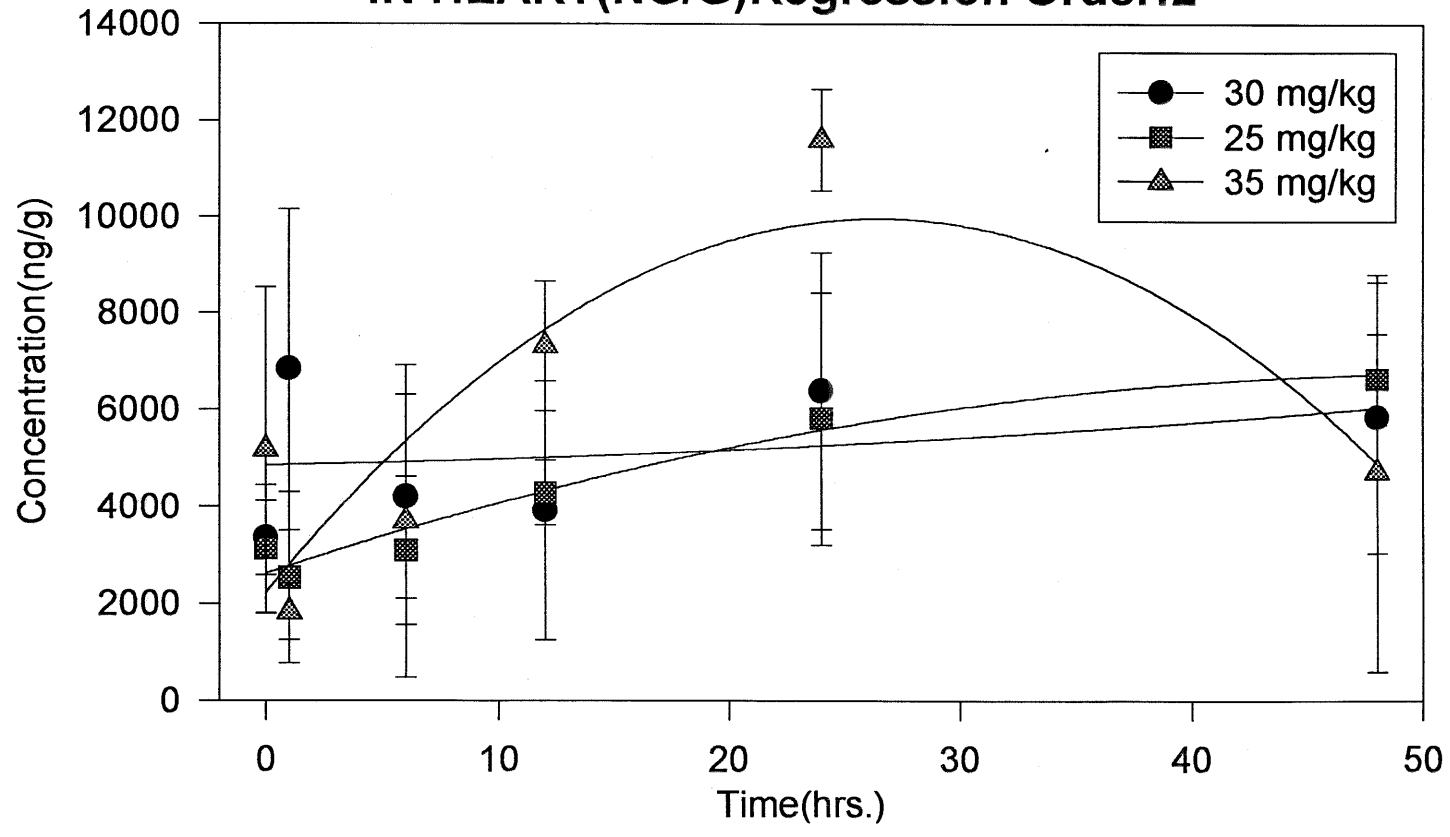
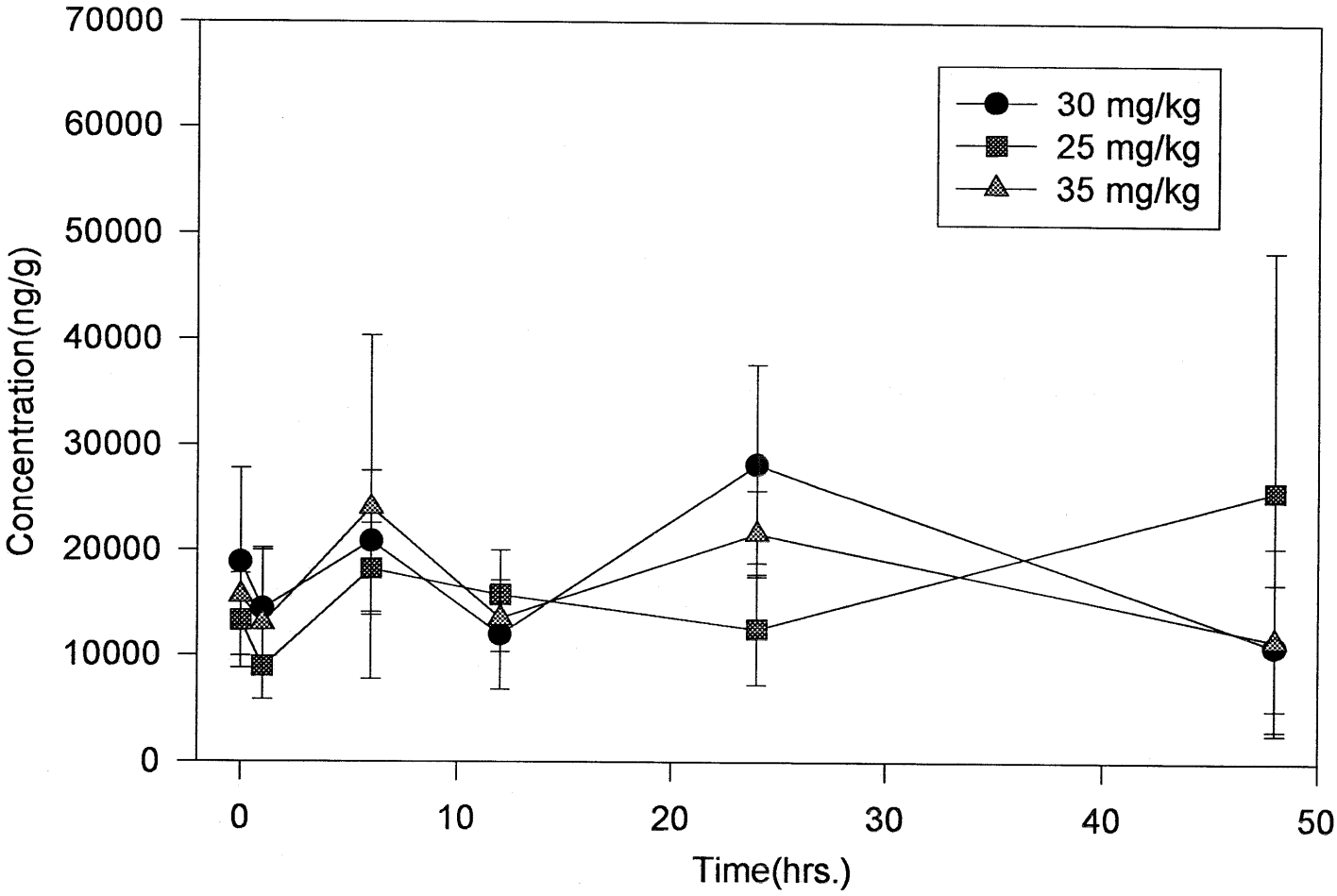


FIGURE 50. Atenolol concentration in kidney (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

ATENOLOL CONCENTRATION IN KIDNEY(NG/G)



ATENOLOL CONCENTRATION IN KIDNEY(NG/G)Regression Order:2

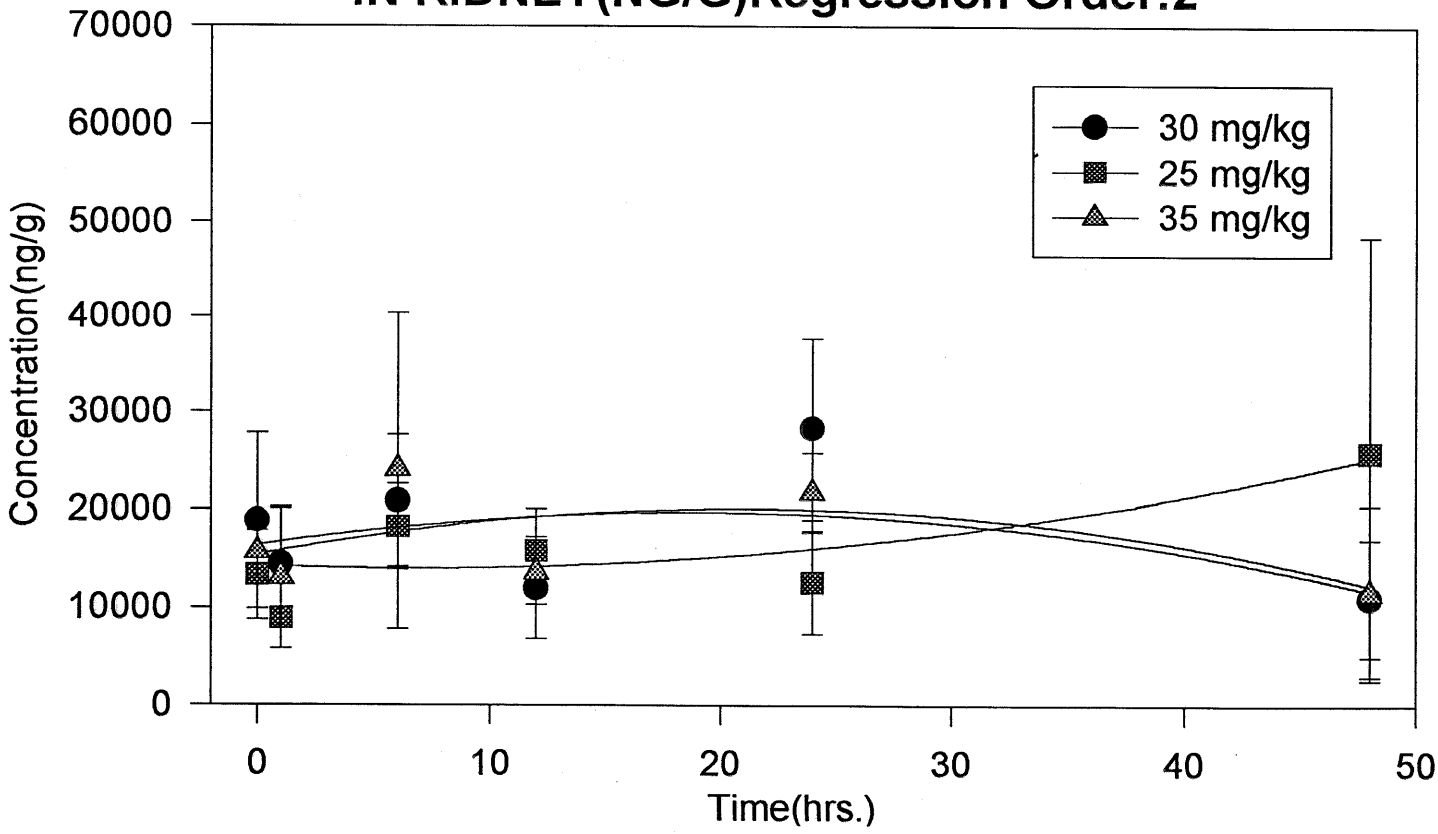
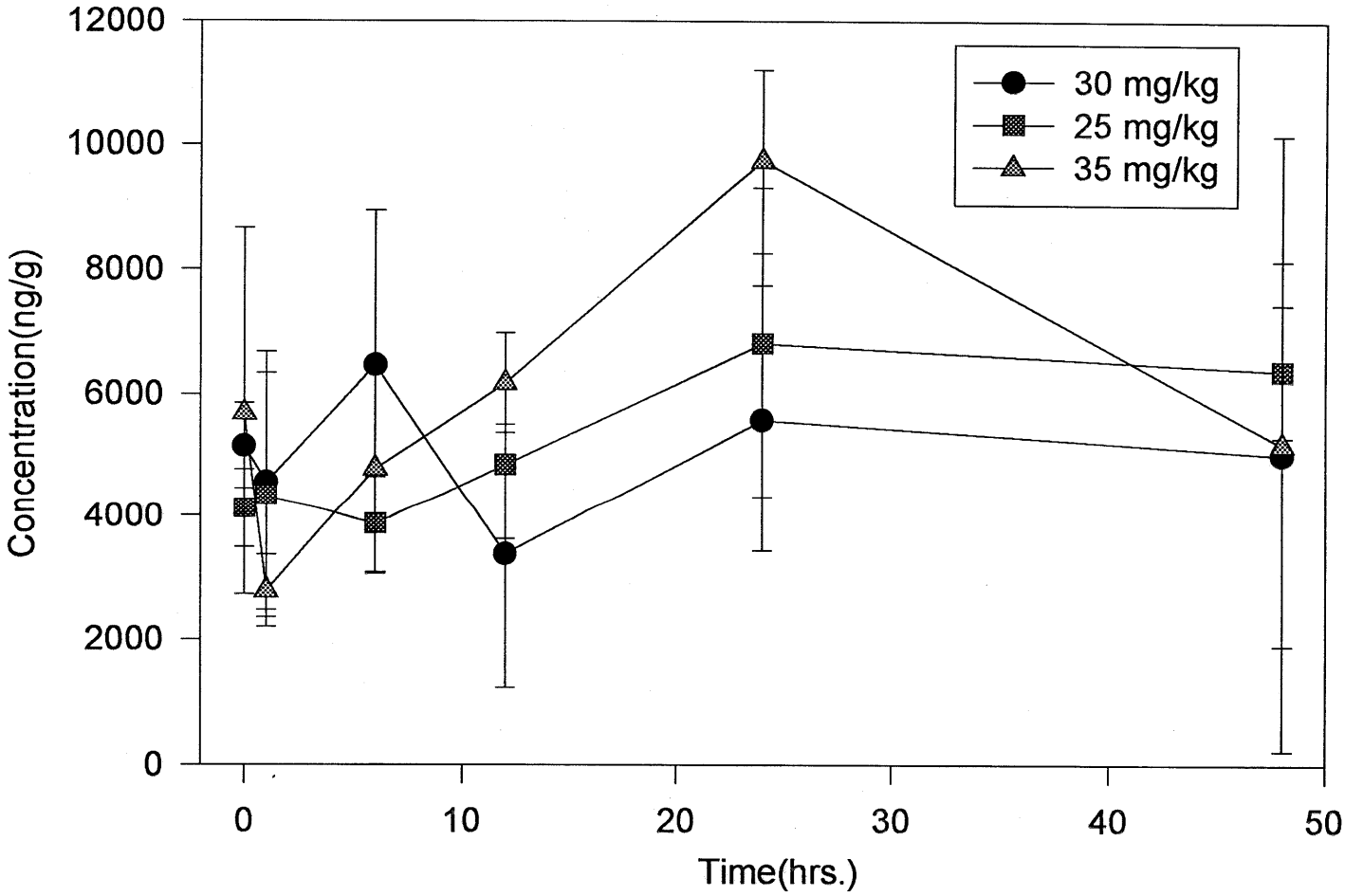


FIGURE 51. Atenolol concentration in liver (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

ATENOLOL CONCENTRATION IN LIVER(NG/G)



ATENOLOL CONCENTRATION IN LIVER(NG/G)Regression Order:2

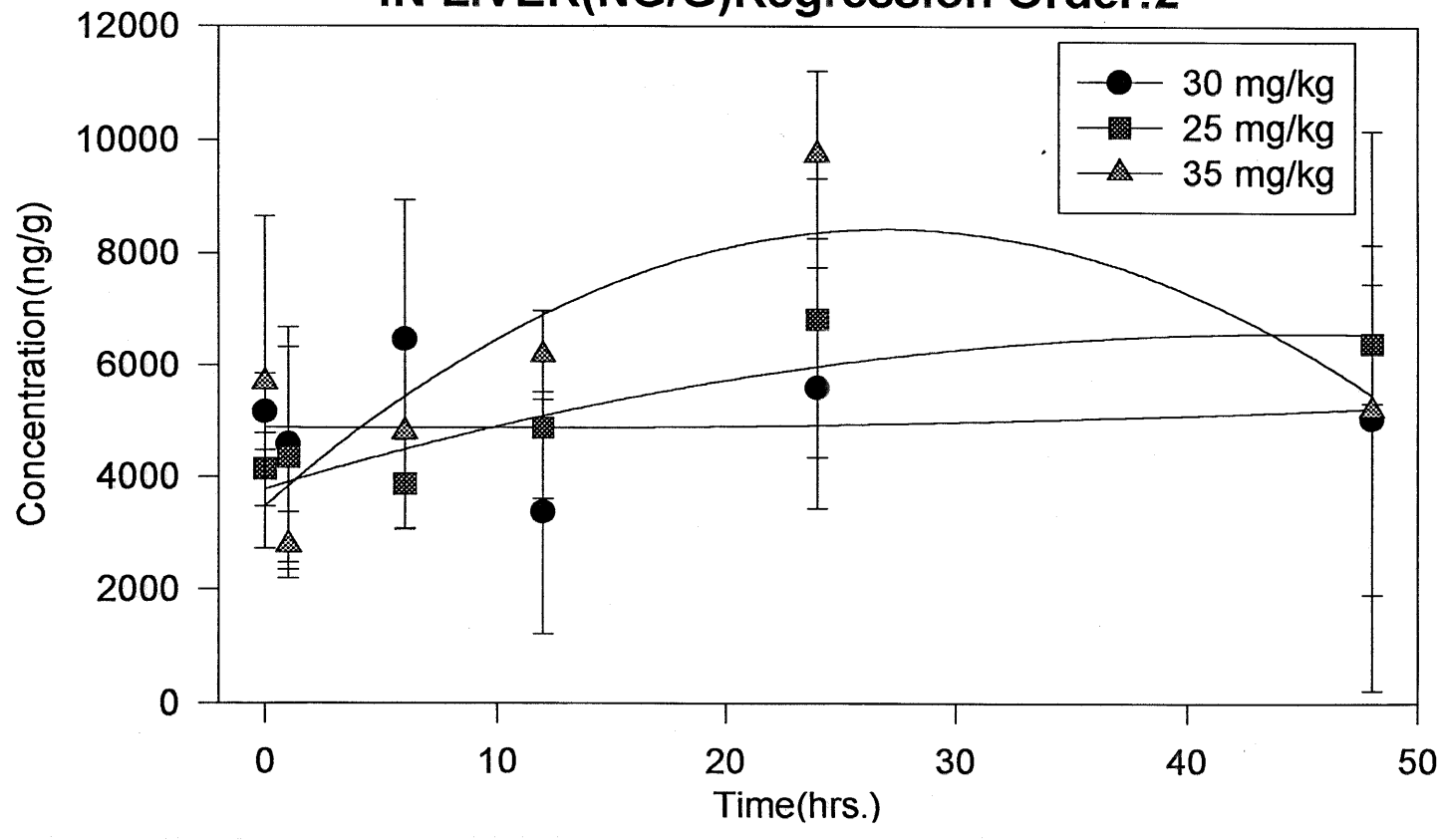
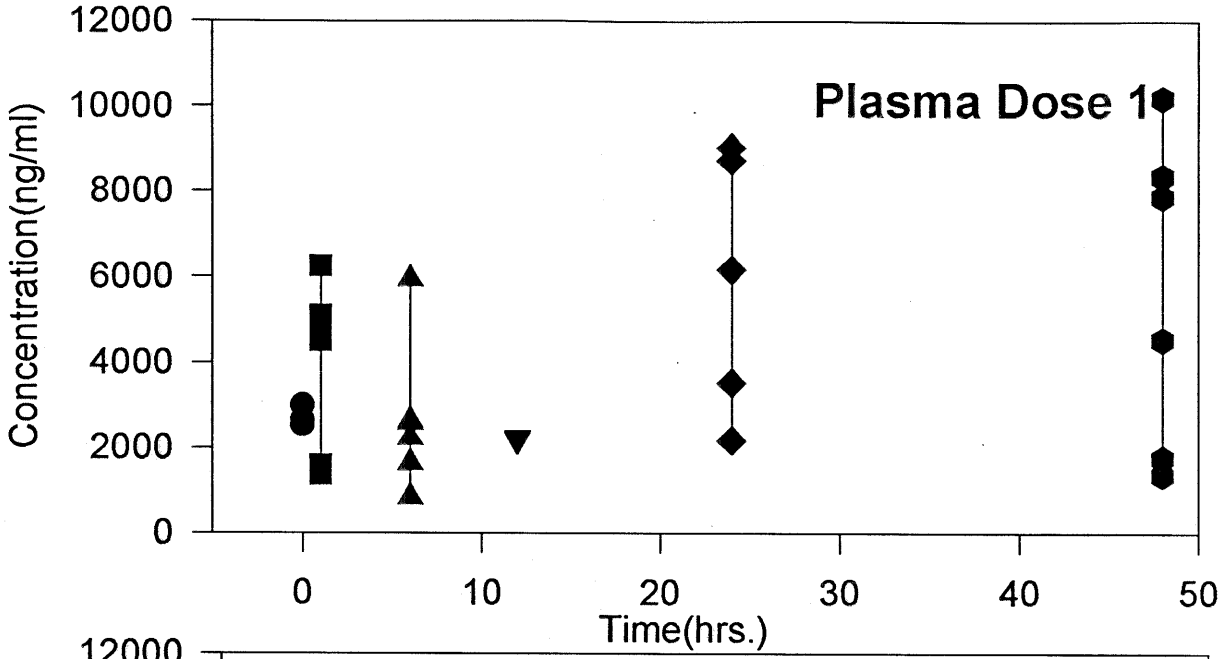
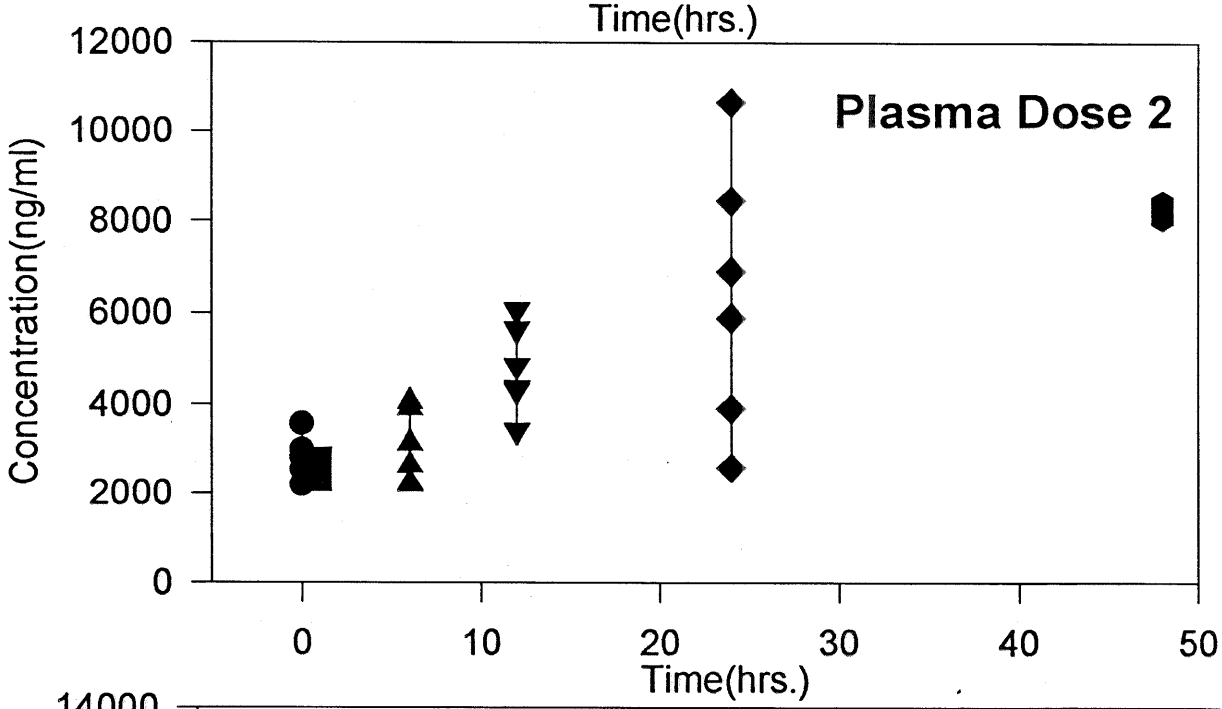


FIGURE 52. Plasma data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three atenolol dosages (dose 1 = 30 mg/kg, dose 2 = 25 mg/kg, and dose 3 = 35 mg/kg) (figure on next page)


Plasma Dose 1
Concentration(ng/ml)
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)


Plasma Dose 2
Concentration(ng/ml)
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

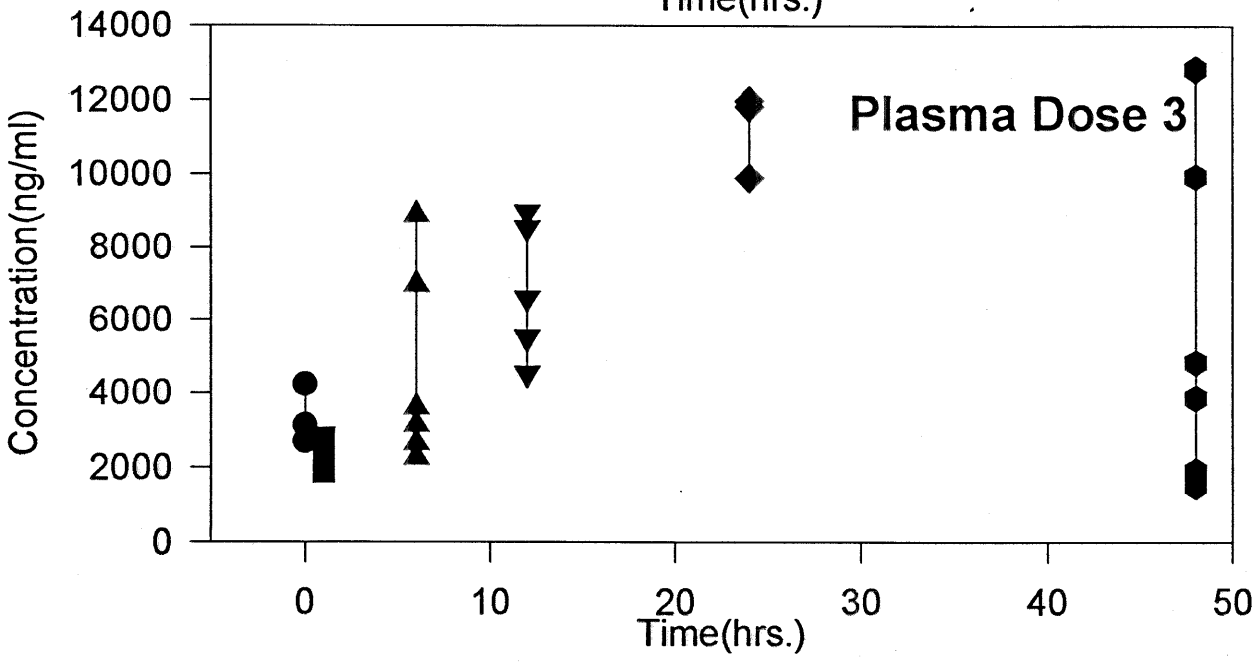
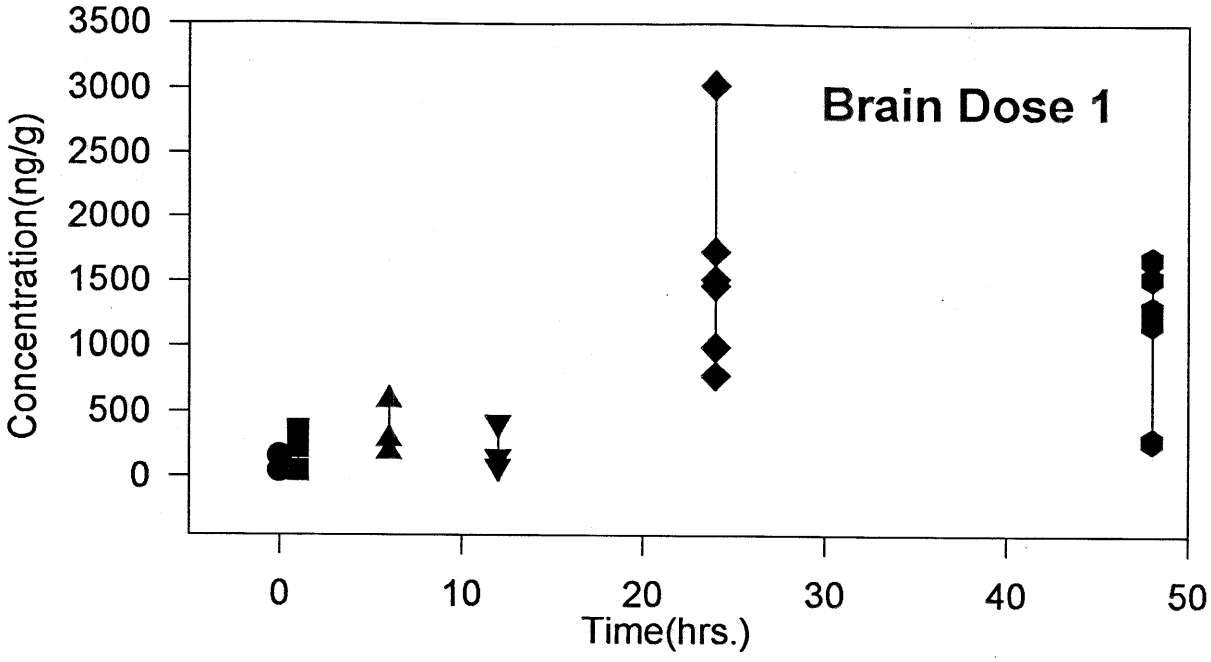
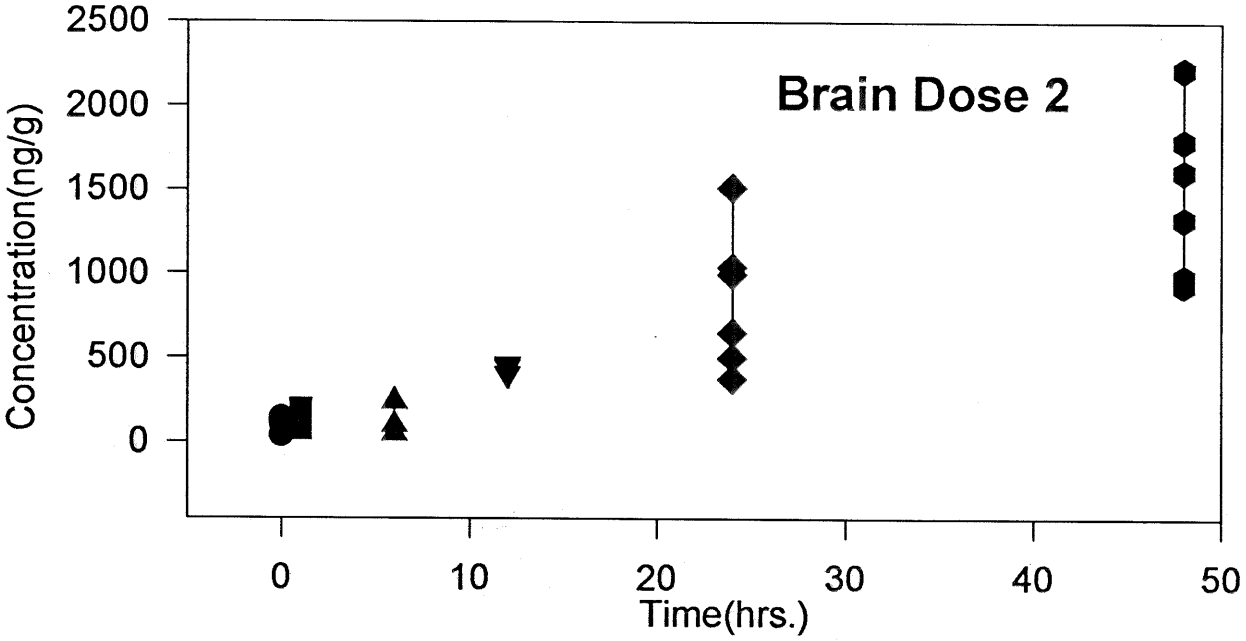

Plasma Dose 3
Concentration(ng/ml)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 53. Brain data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three atenolol dosages (dose 1 = 30 mg/kg, dose 2 = 25 mg/kg, and dose 3 = 35 mg/kg) (figure on next page)


Brain Dose 1
Concentration(ng/g)
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)


Brain Dose 2
Concentration(ng/g)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)

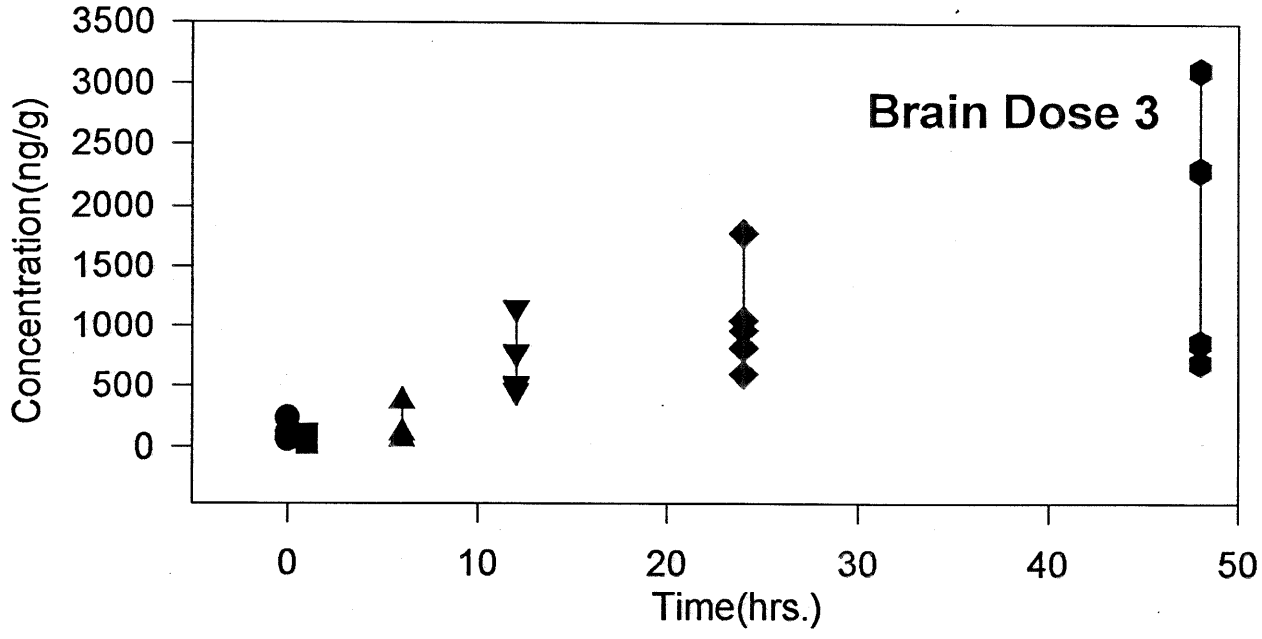
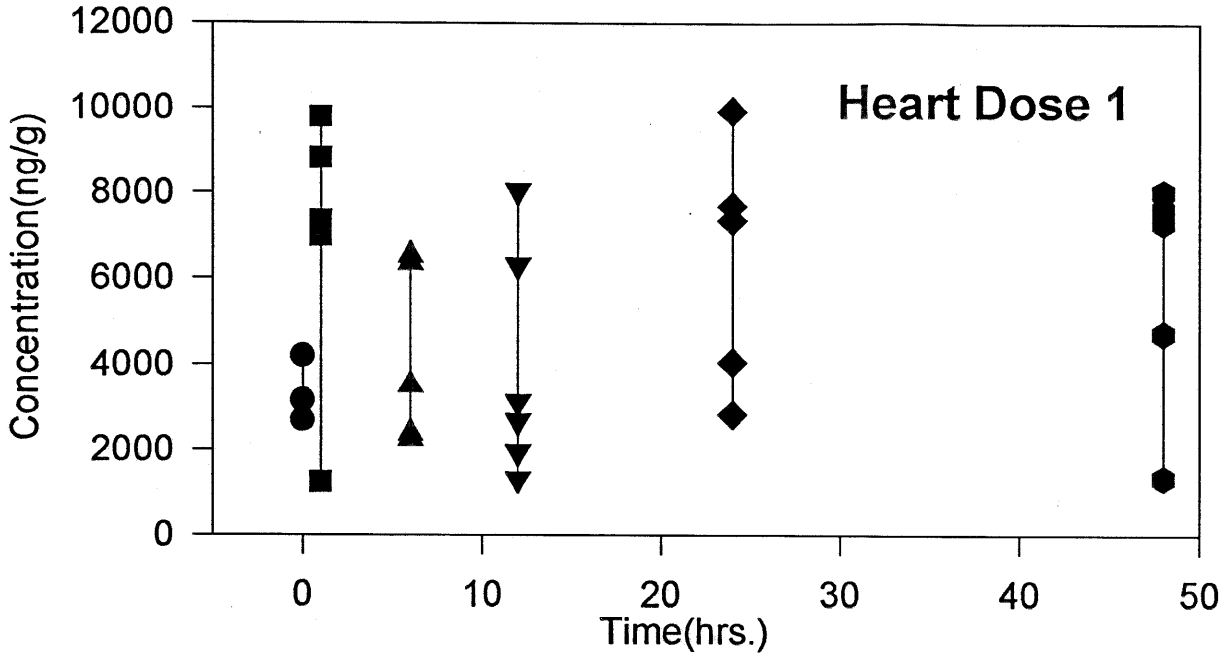
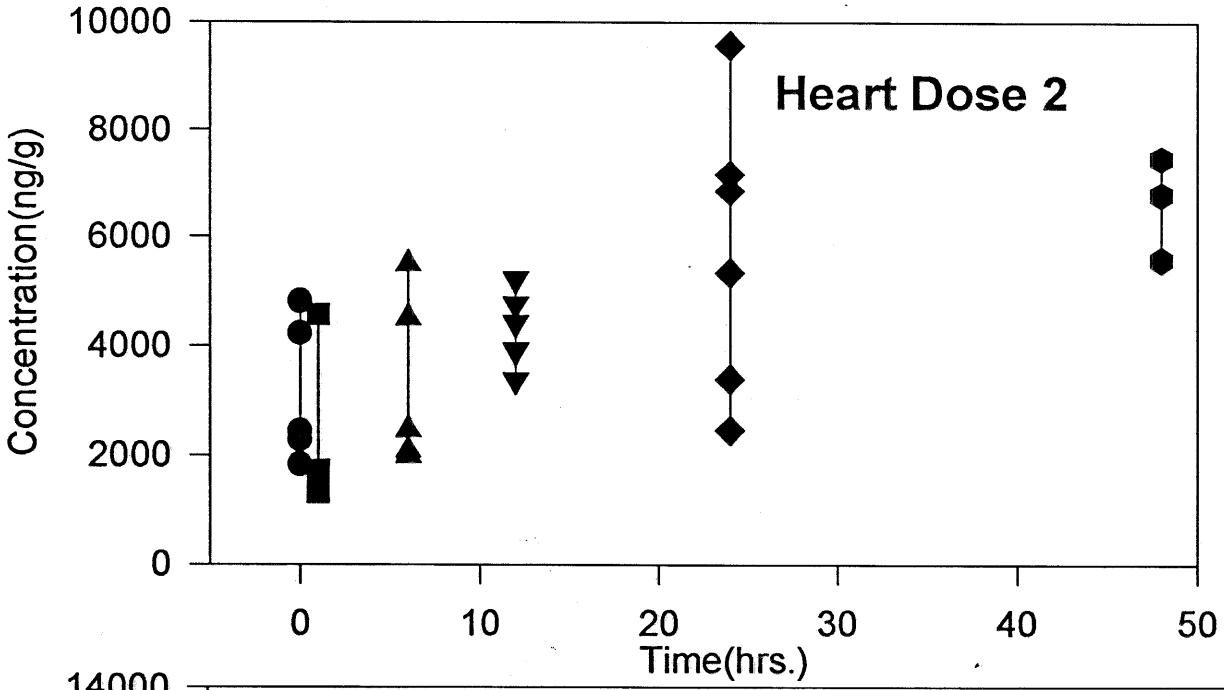

Brain Dose 3
Concentration(ng/g)
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 54. Heart data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three atenolol dosages (dose 1 = 30 mg/kg, dose 2 = 25 mg/kg, and dose 3 = 35 mg/kg) (figure on next page)


Heart Dose 1
Concentration(ng/g)
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)


Heart Dose 2
Concentration(ng/g)
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

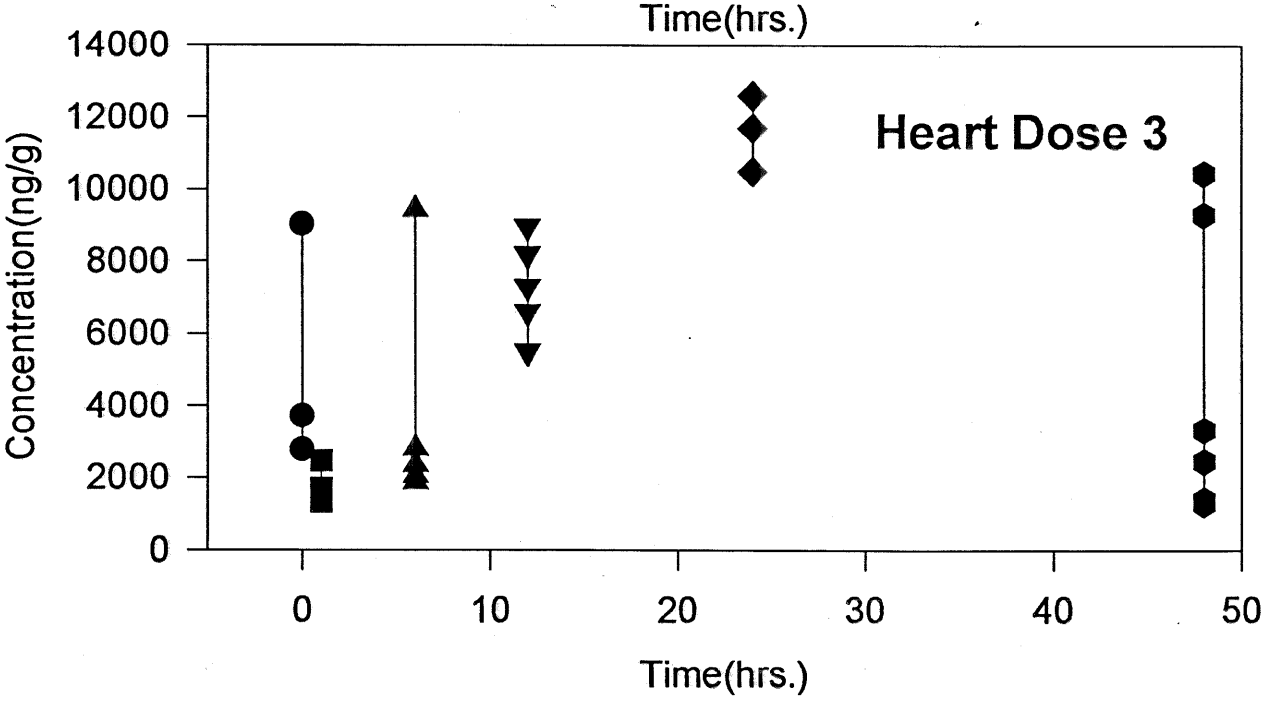
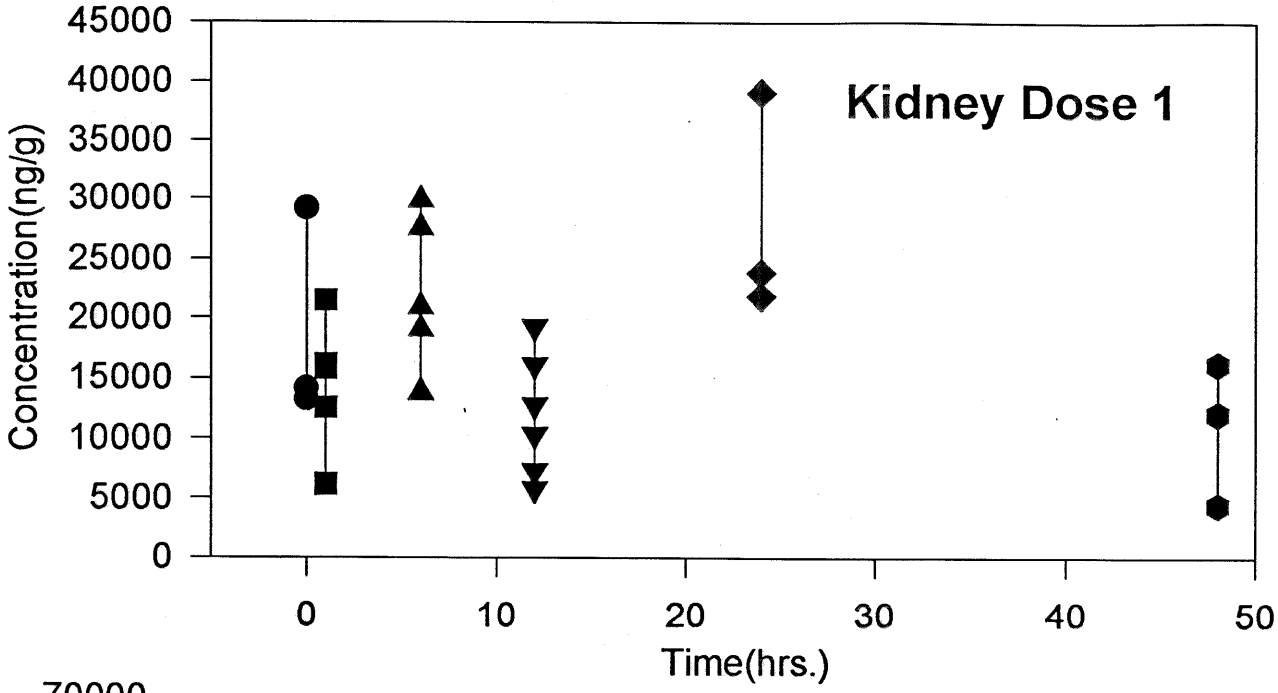
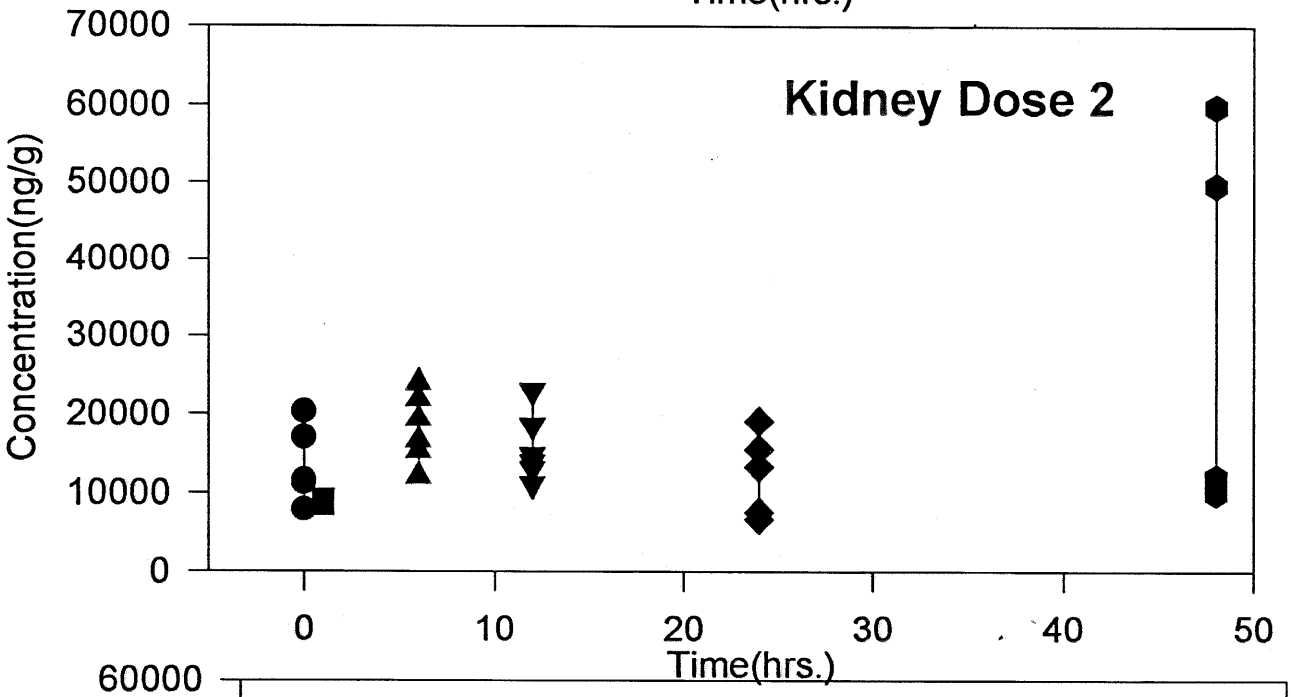

Heart Dose 3
Concentration(ng/g)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 55. Kidney data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three atenolol dosages (dose 1 = 30 mg/kg, dose 2 = 25 mg/kg, and dose 3 = 35 mg/kg)
(figure on next page)


Kidney Dose 1
Concentration(ng/g)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
Time(hrs.)


Kidney Dose 2
Concentration(ng/g)
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
Time(hrs.)

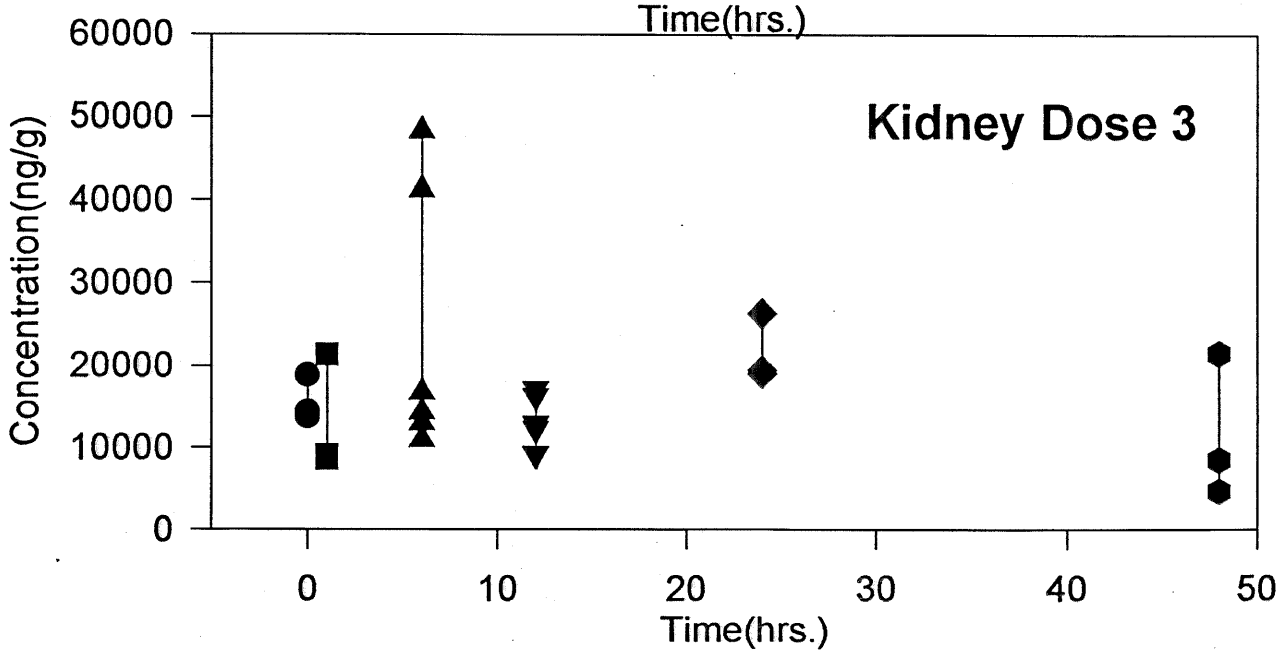
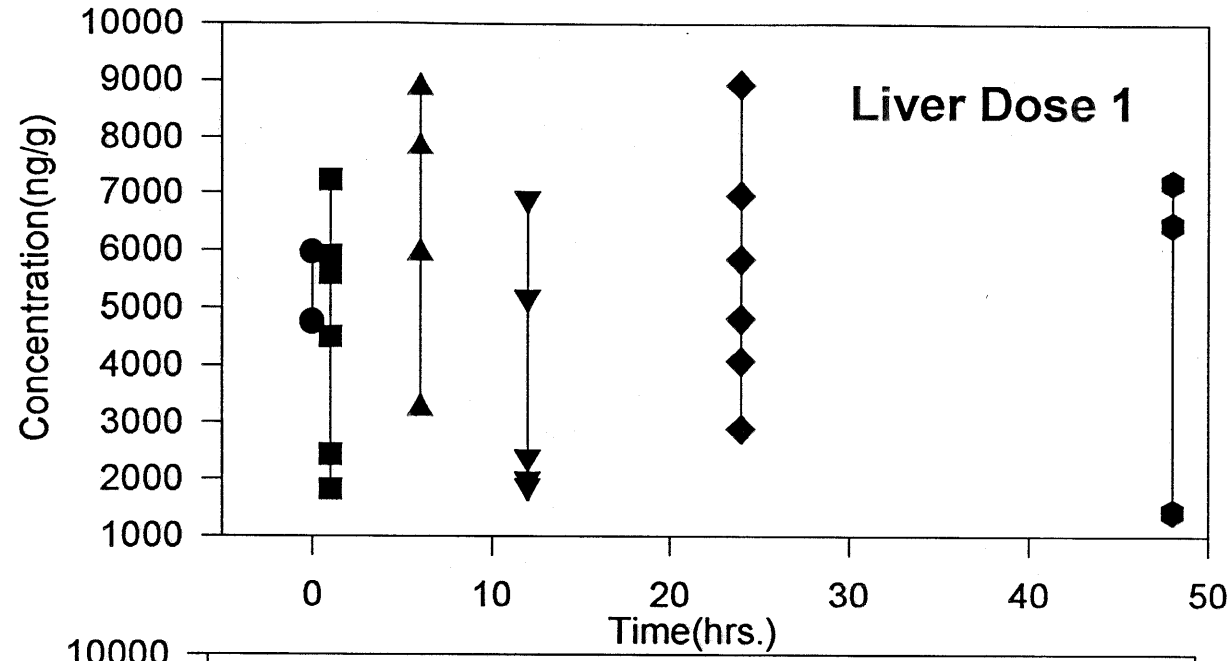
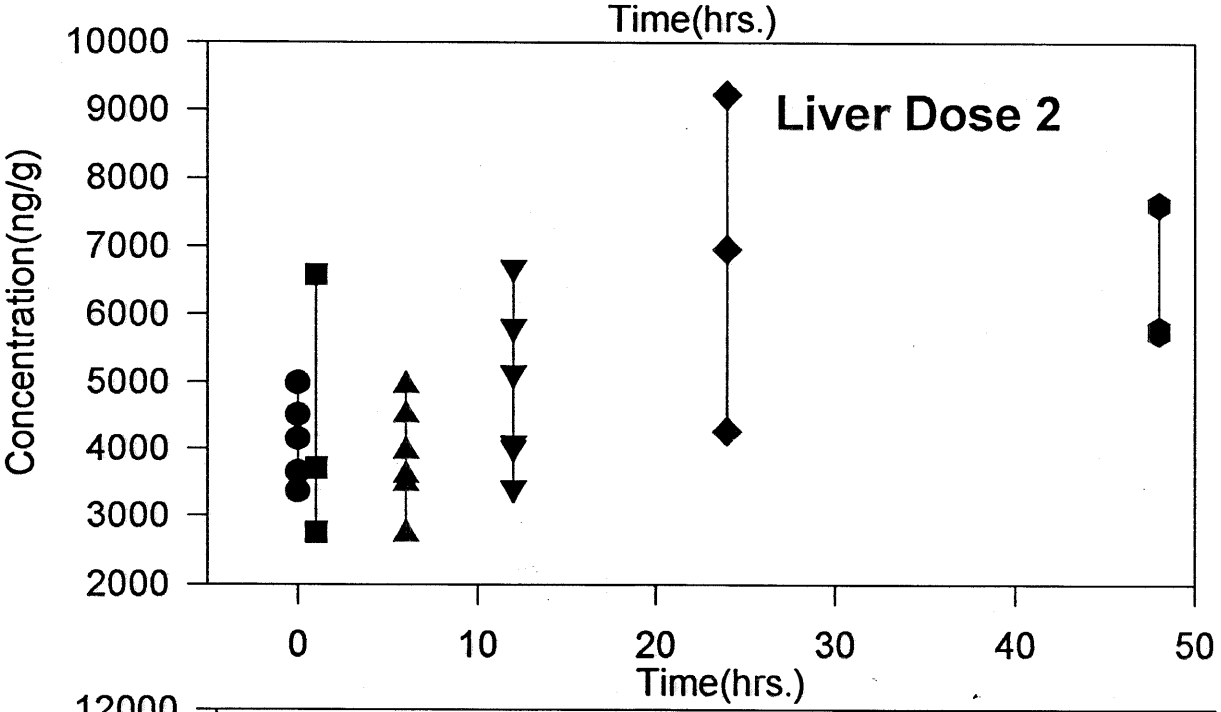
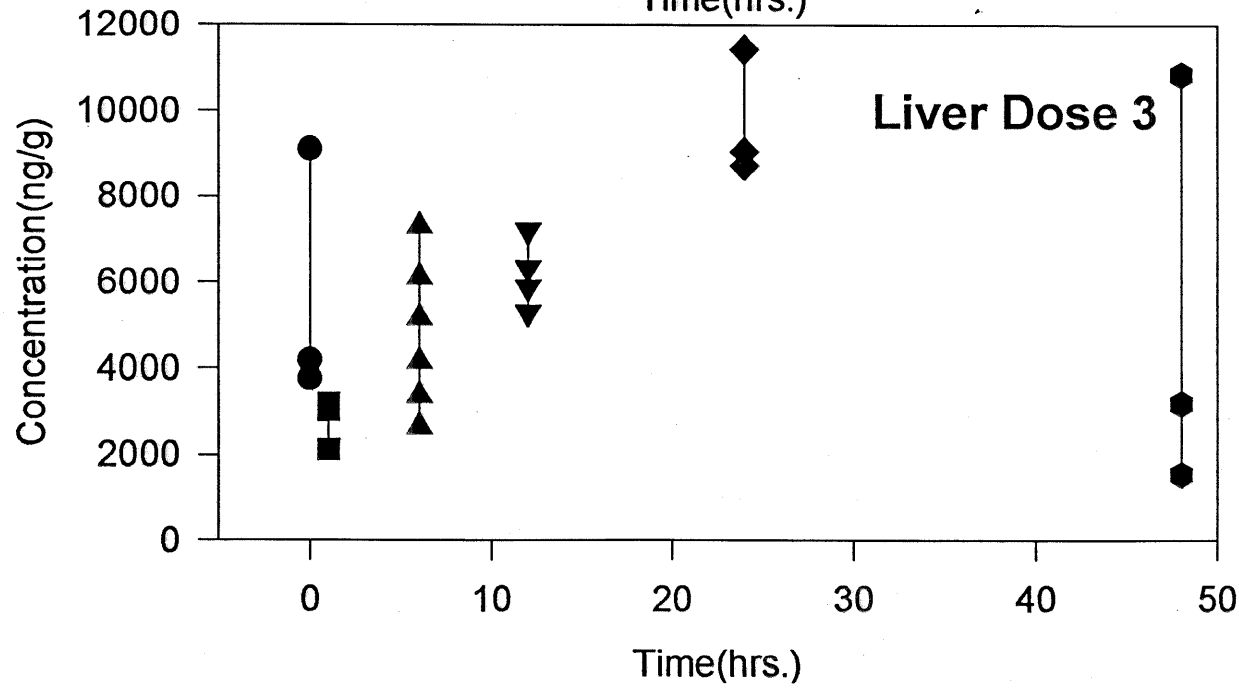

Kidney Dose 3
Concentration(ng/g)
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 56. Liver data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three atenolol dosages (dose 1 = 30 mg/kg, dose 2 = 25 mg/kg, and dose 3 = 35 mg/kg) (figure on next page)


Liver Dose 1
Concentration(ng/g)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10
20
30
40
50
Time(hrs.)


Liver Dose 2
Concentration(ng/g)
10000
9000
8000
7000
6000
5000
4000
3000
2000
0
10
20
30
40
50
Time(hrs.)


Liver Dose 3
Concentration(ng/g)
12000
10000
8000
6000
4000
2000
0
10
20
30
40
50
Time(hrs.)

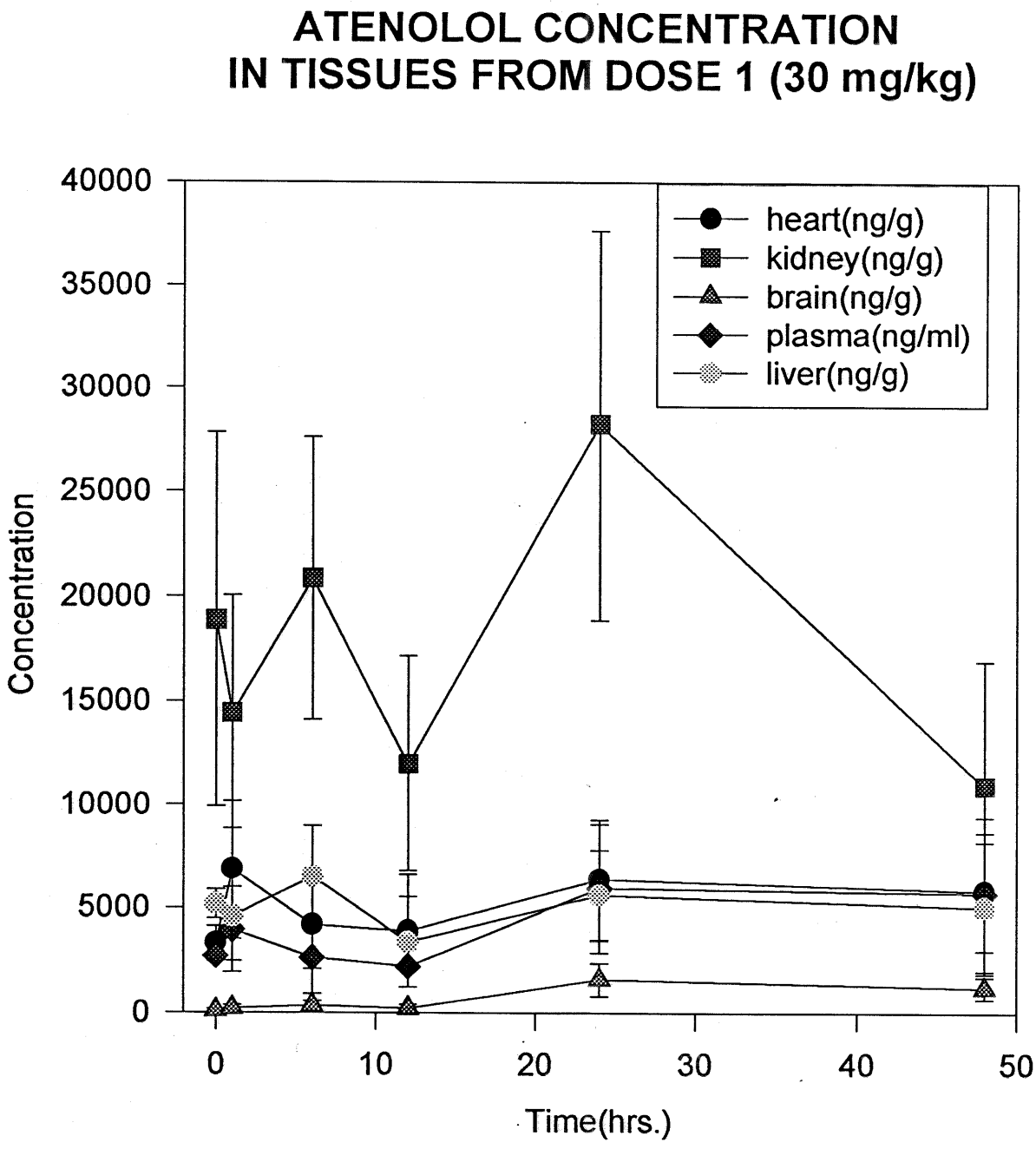


FIGURE 57. Point-to-point analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 30 mg/kg dose

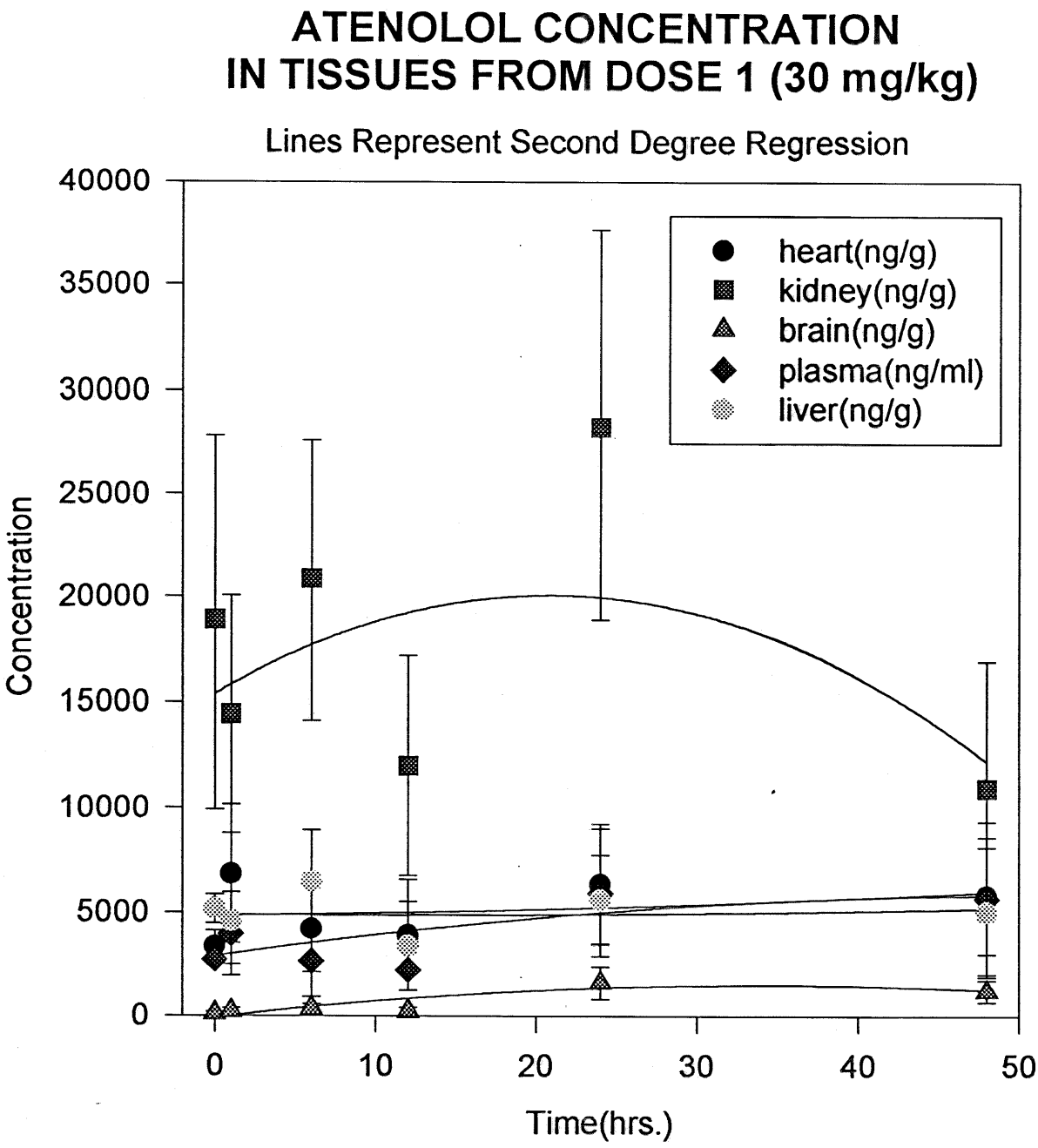


FIGURE 58. Second-order regression analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 30 mg/kg dose

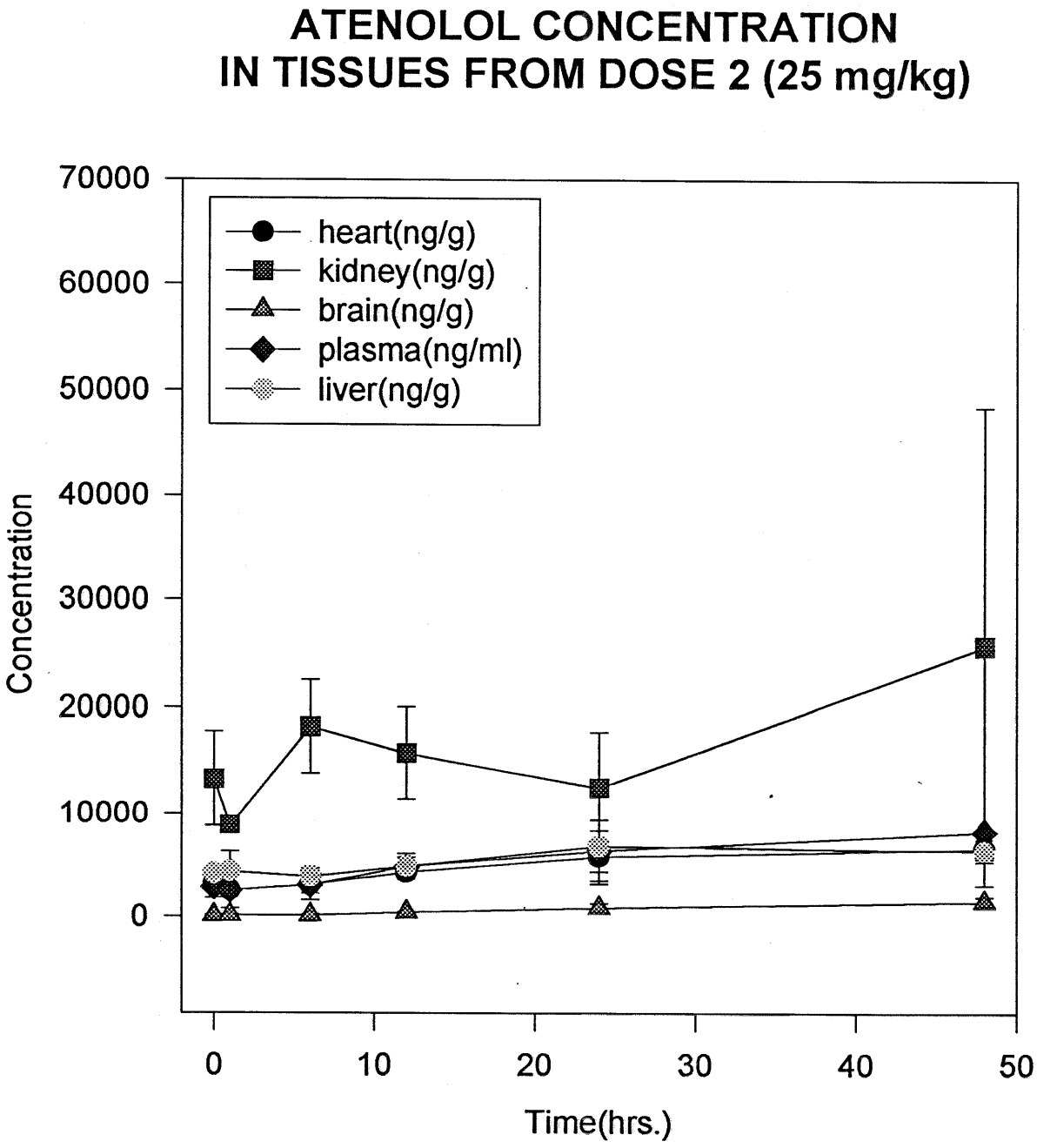


FIGURE 59. Point-to-point analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 25 mg/kg dose

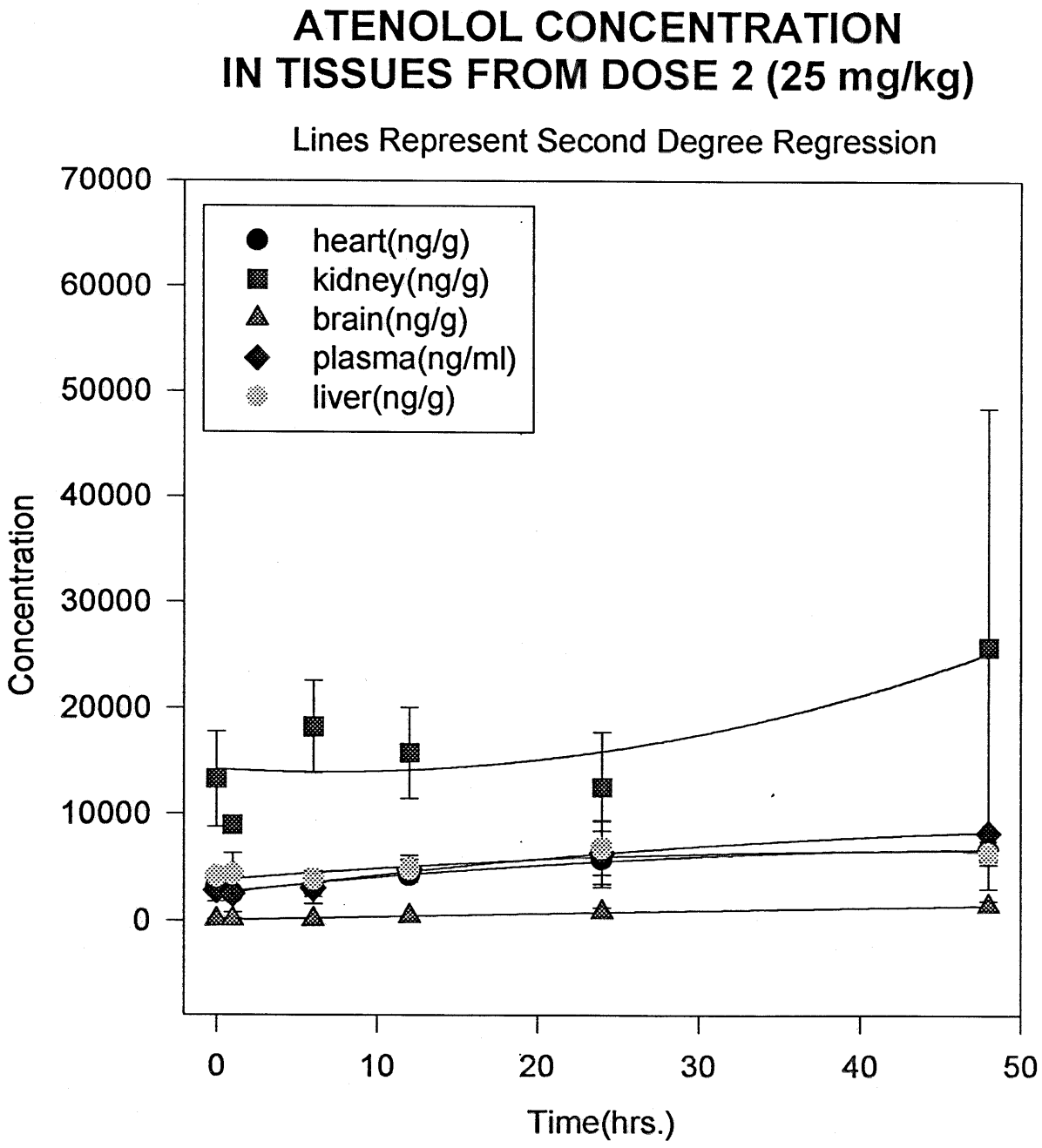


FIGURE 60. Second-order regression analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 25 mg/kg dose

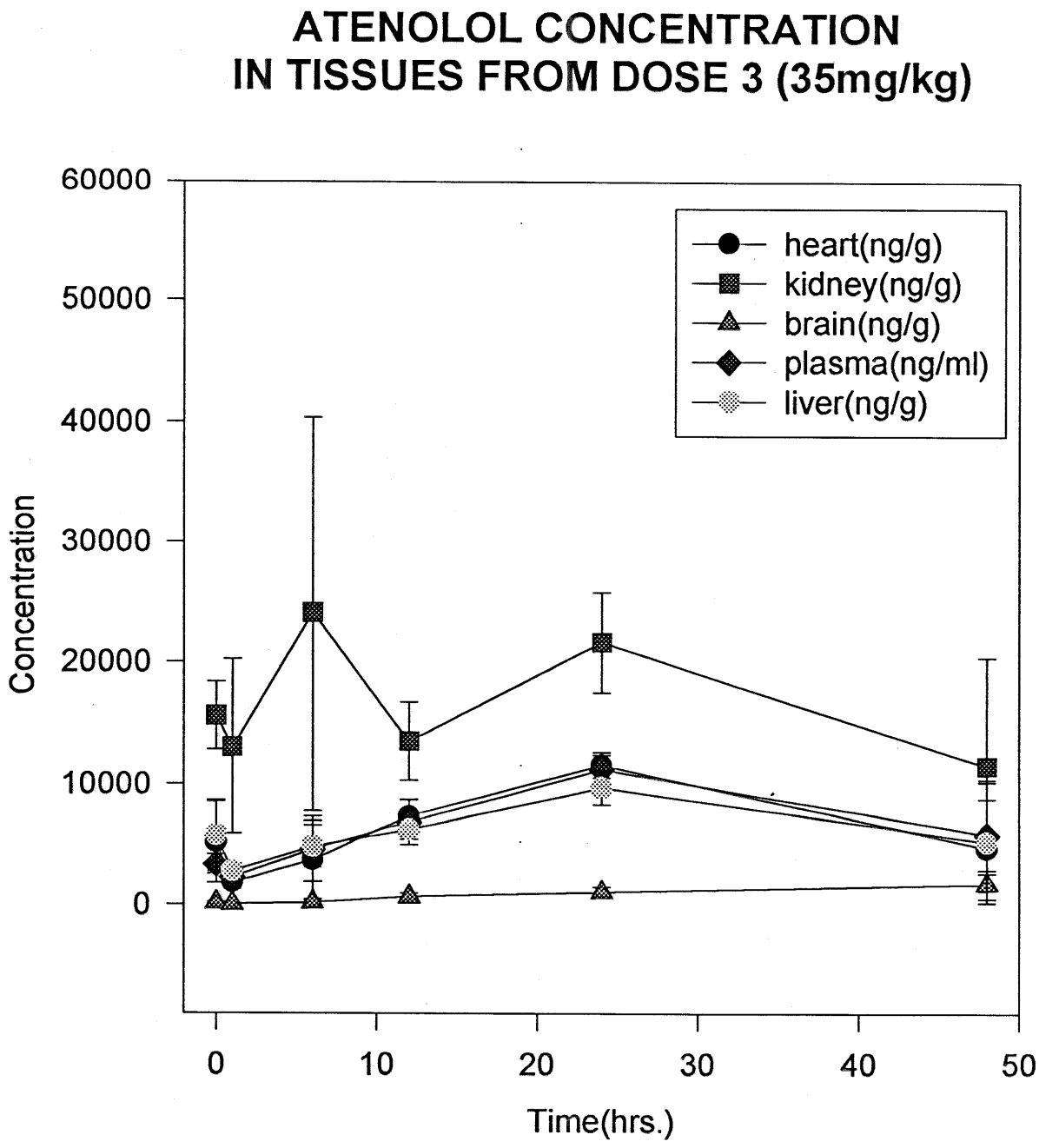


FIGURE 61. Point-to-point analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 35 mg/kg dose

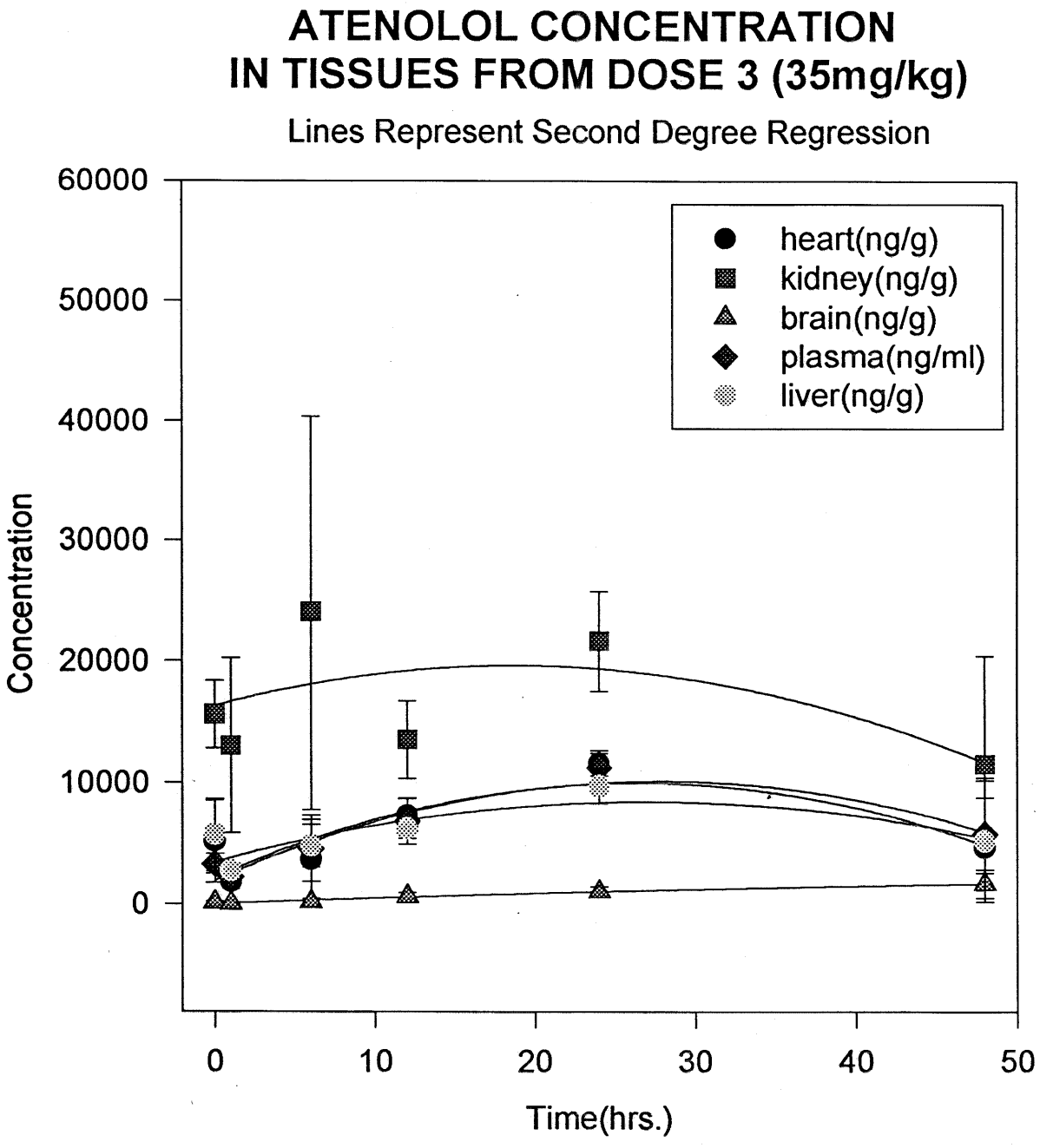
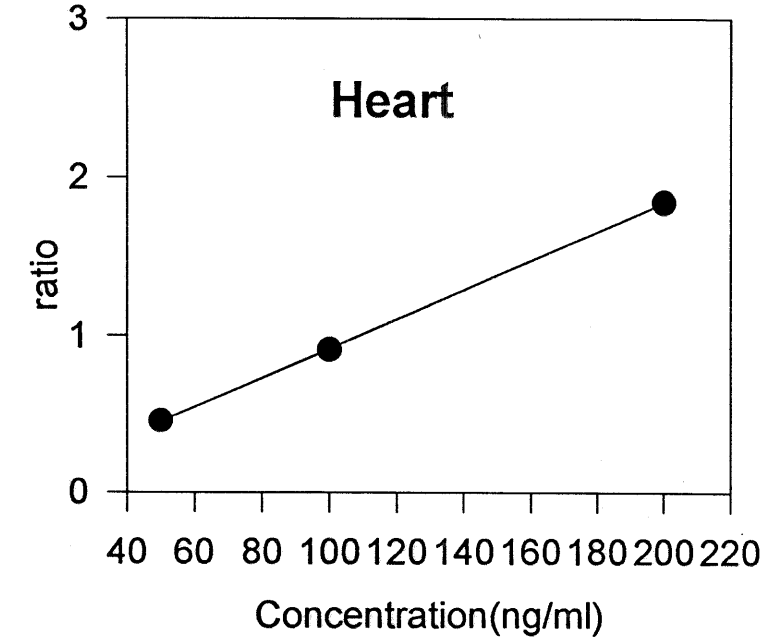
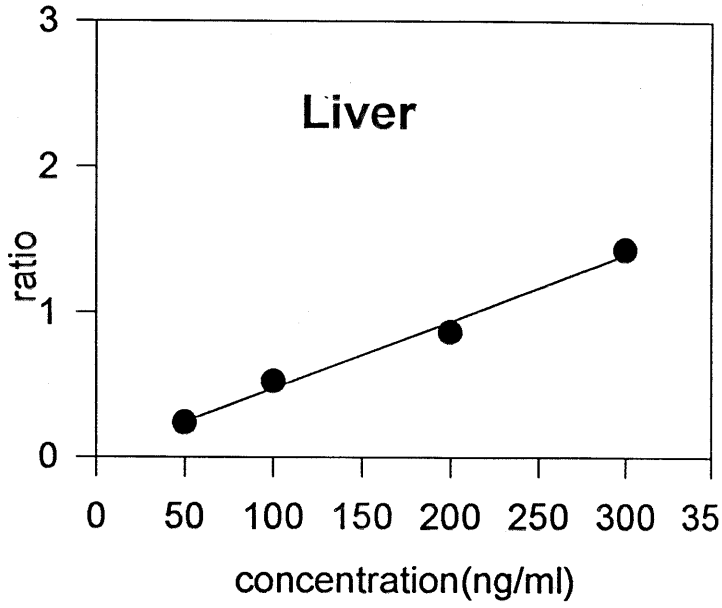
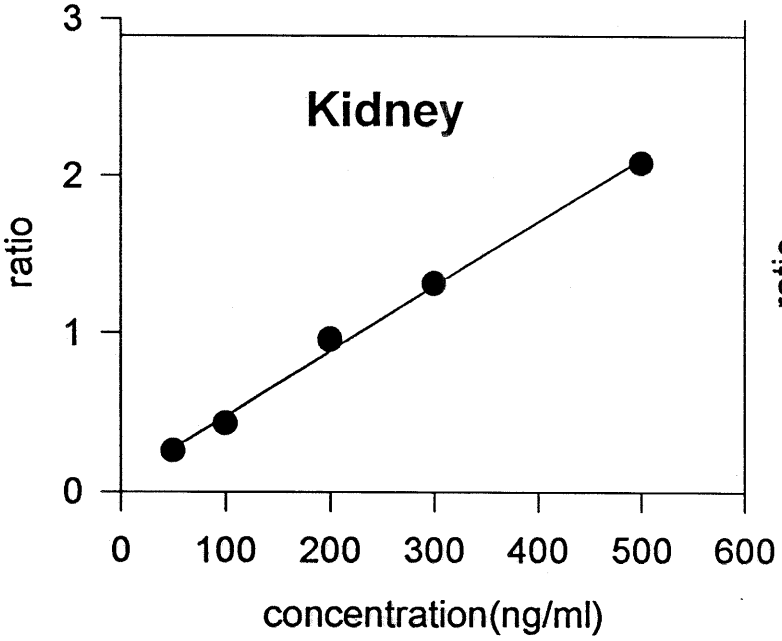
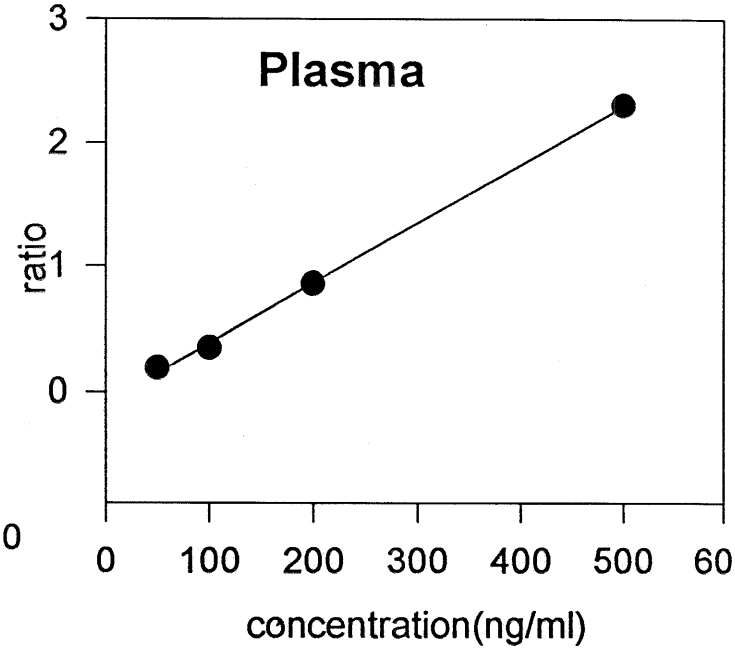


FIGURE 62. Second-order regression analysis of atenolol concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 35 mg/kg dose









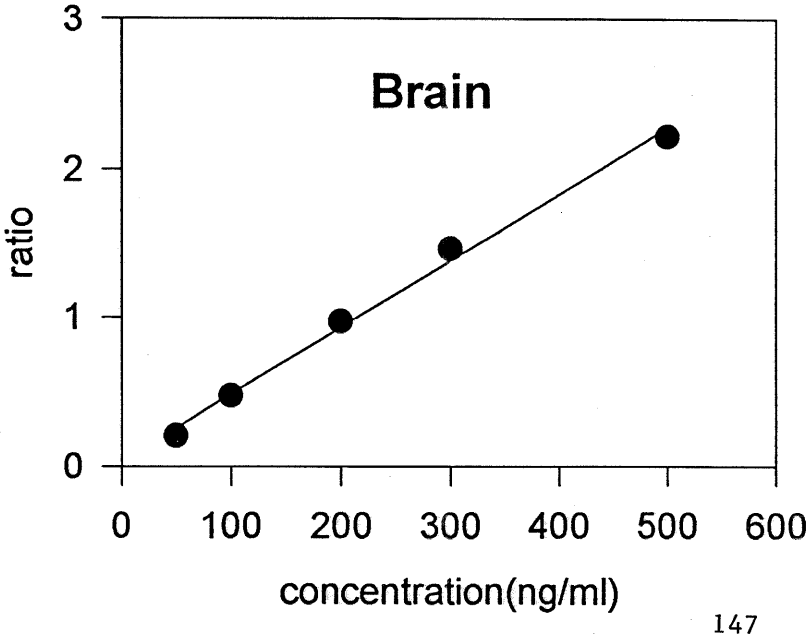
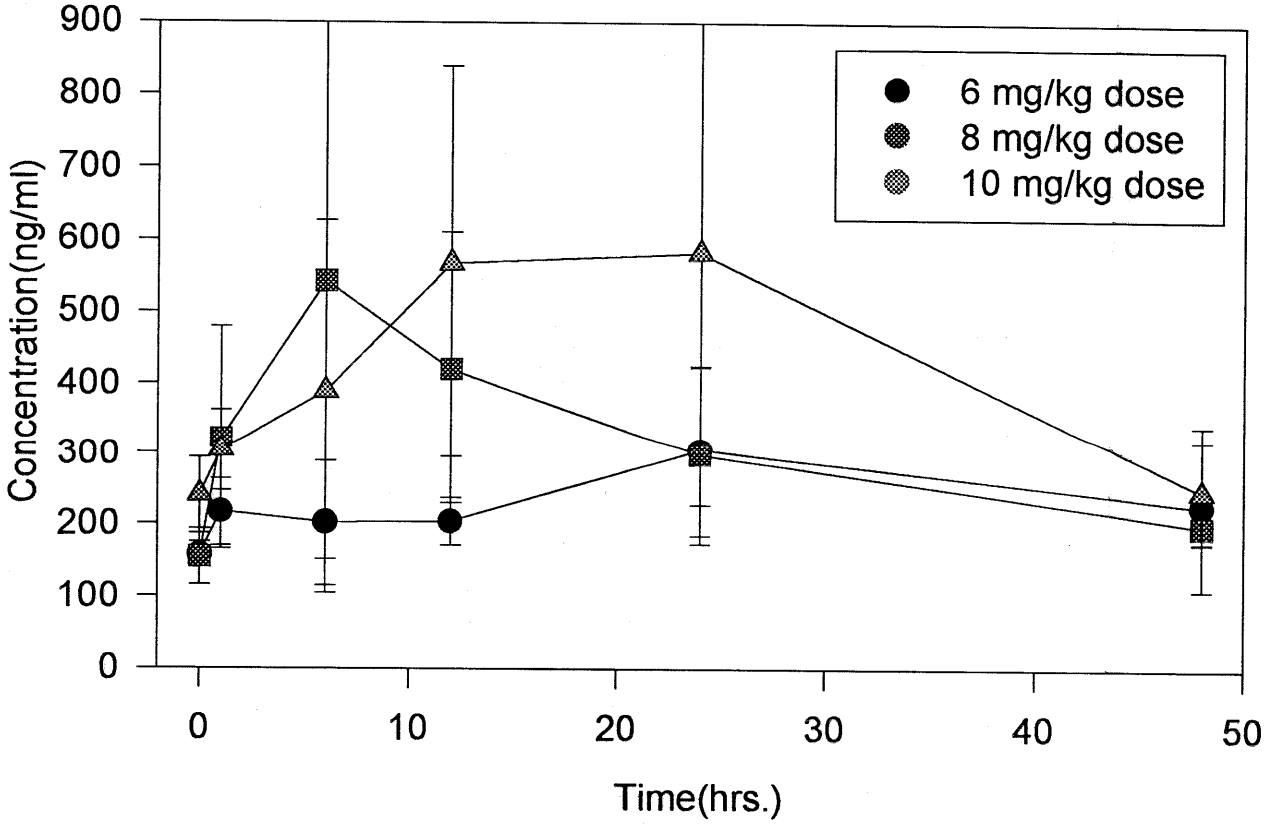


FIGURE 63. Graphs of standard curve (concentration vs. the ratio of drug/internal standard) for terfenadine in heart, kidney, brain, liver, and plasma

FIGURE 64. Terfenadine concentration in plasma (ng/ml, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

TERFENADINE CONCENTRATION IN PLASMA(NG/ML)



TERFENADINE CONCENTRATION IN PLASMA(NG/ML)REGRESSION ORDER:2

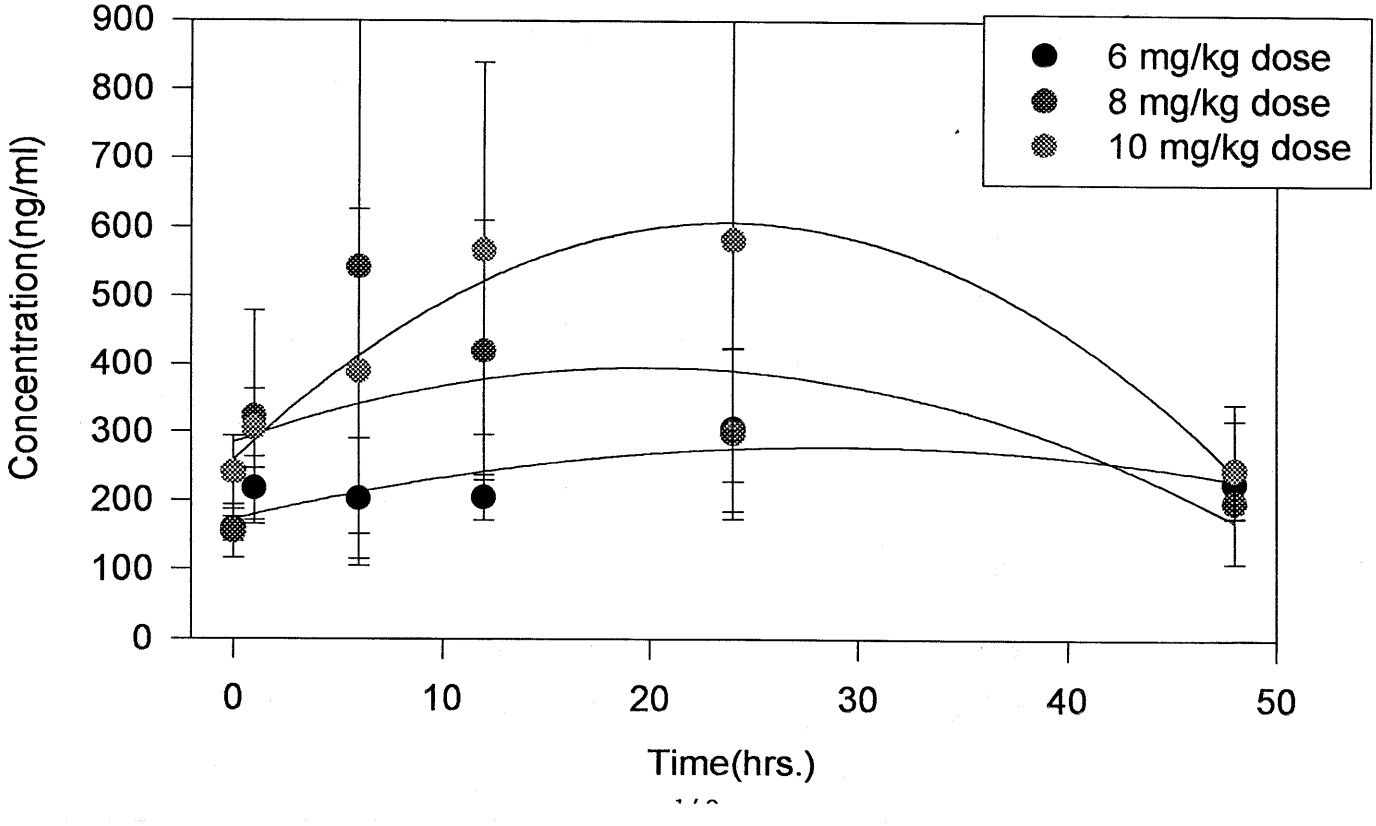
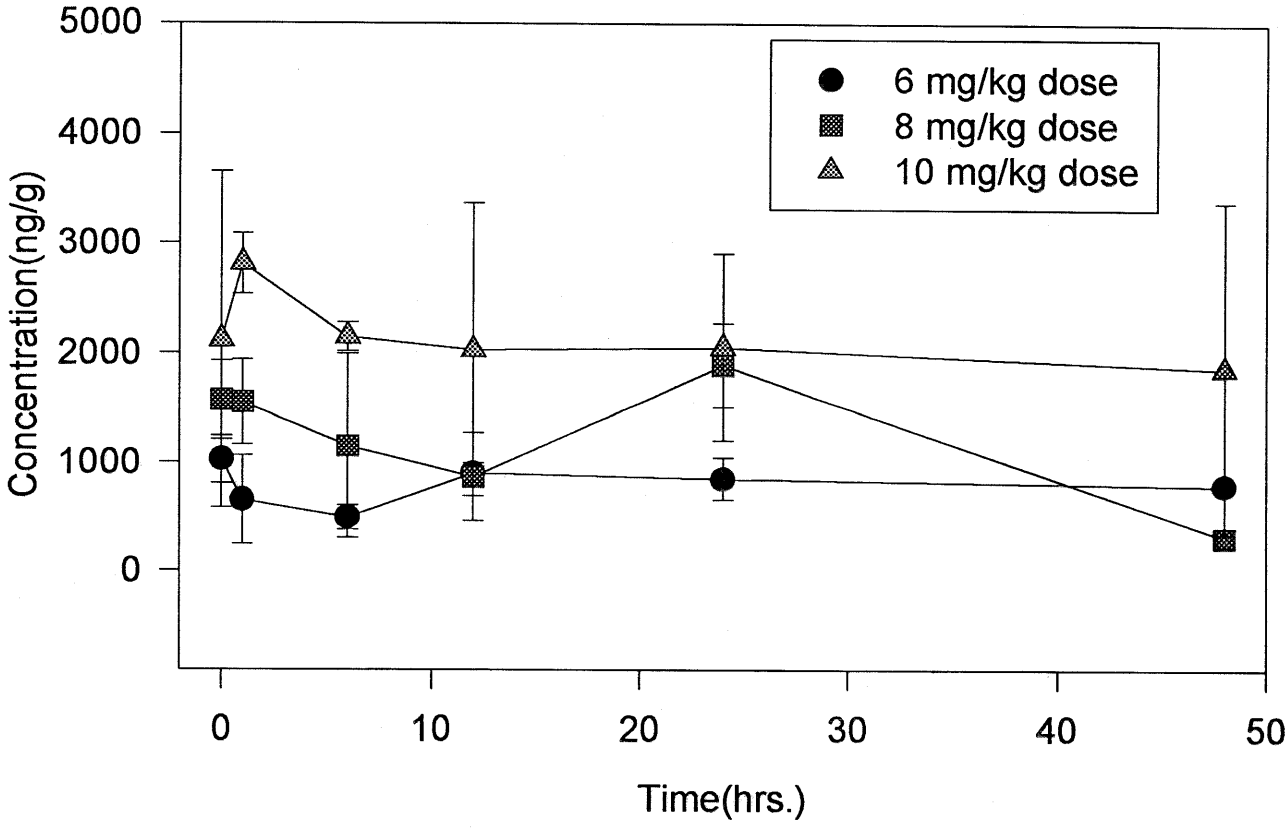


FIGURE 65. Terfenadine concentration in brain (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

TERFENADINE CONCENTRATION IN BRAIN(NG/G)



TERFENADINE CONCENTRATION IN BRAIN(NG/G)REGRESSION ORDER:2

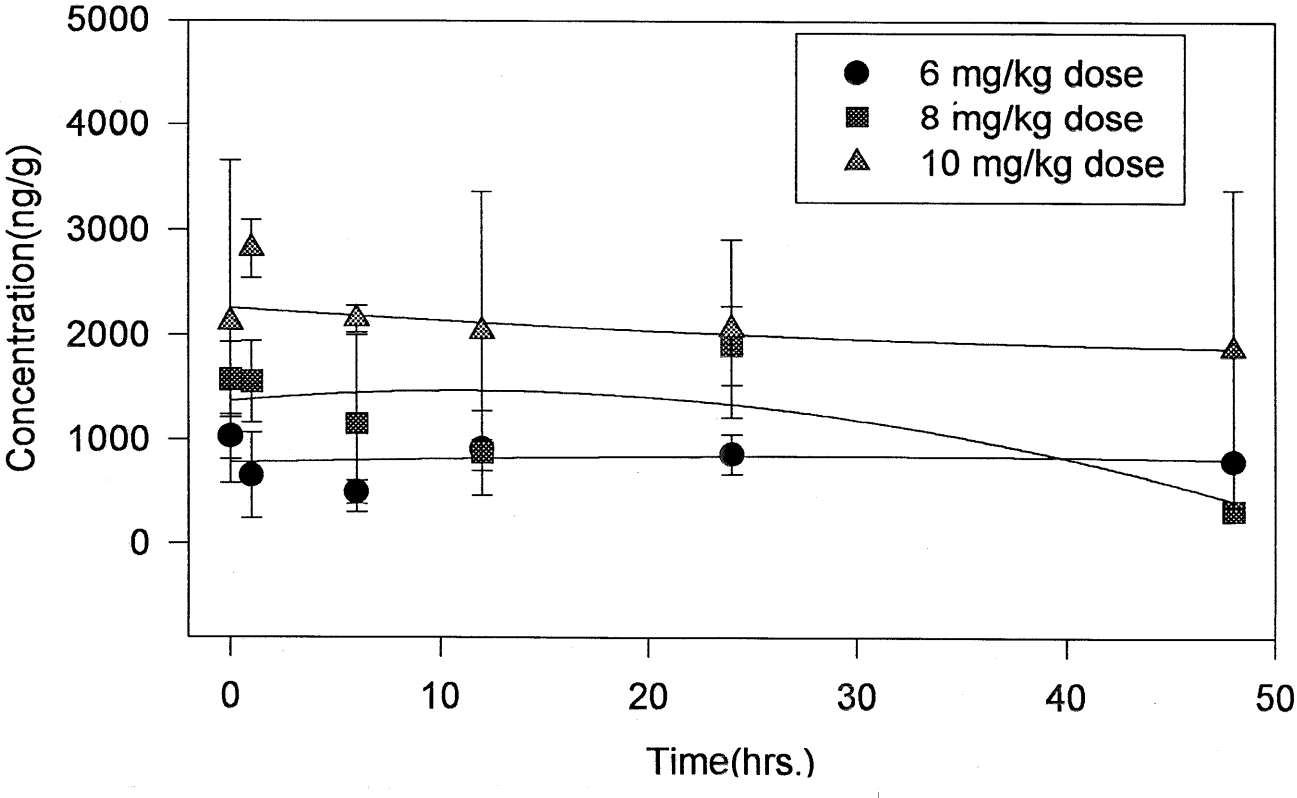
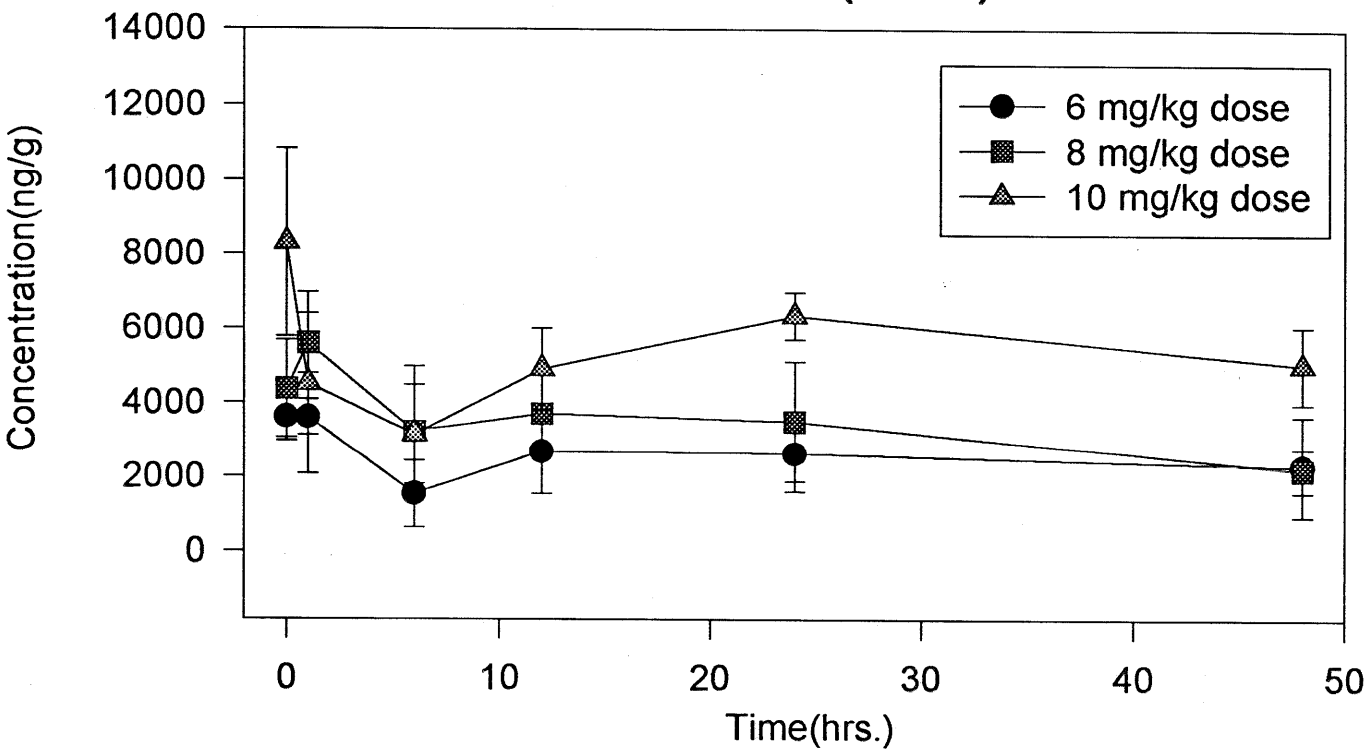


FIGURE 66. Terfenadine concentration in heart (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

TERFENADINE CONCENTRATION IN HEART(NG/G)



TERFENADINE CONCENTRATION IN HEART(NG/G)REGRESSION ORDER:2

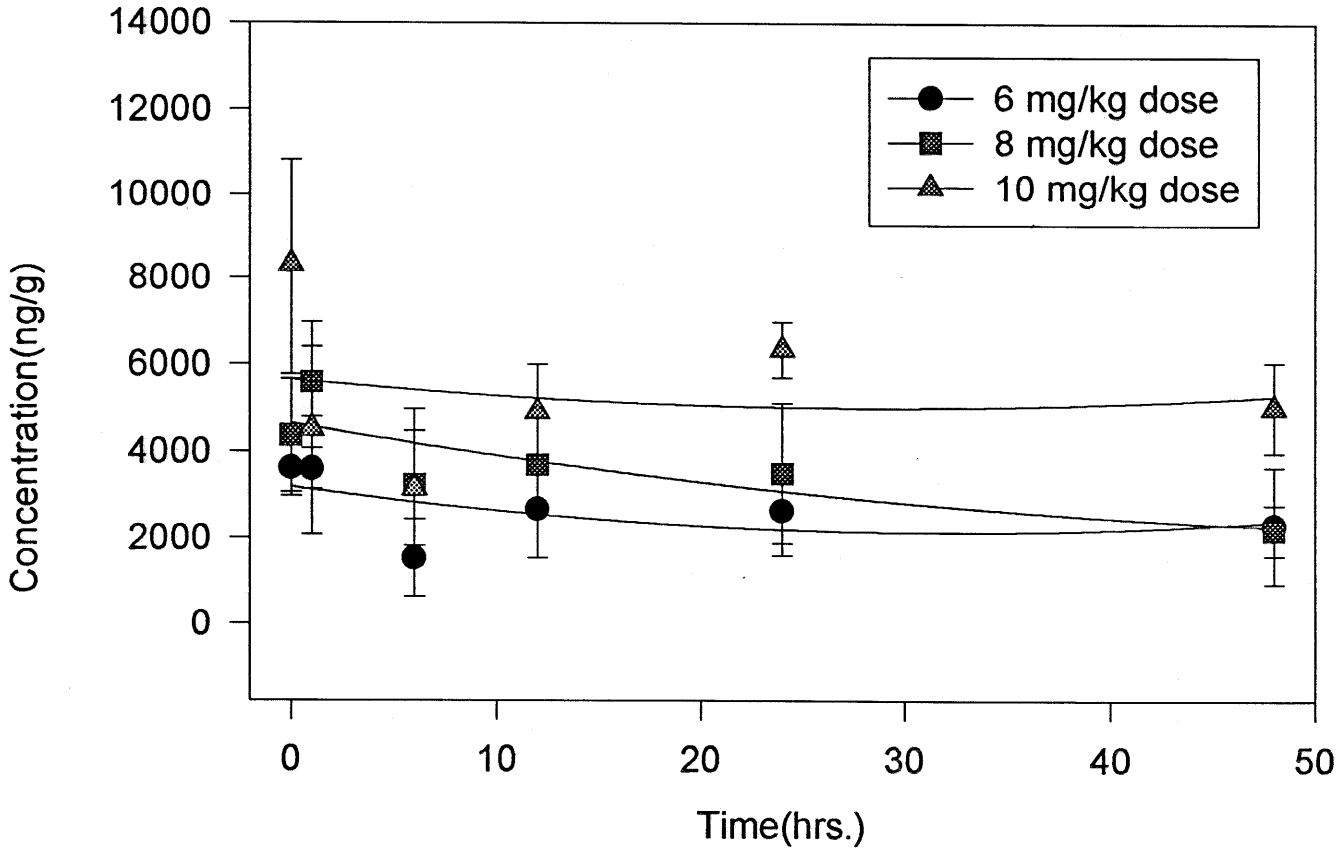
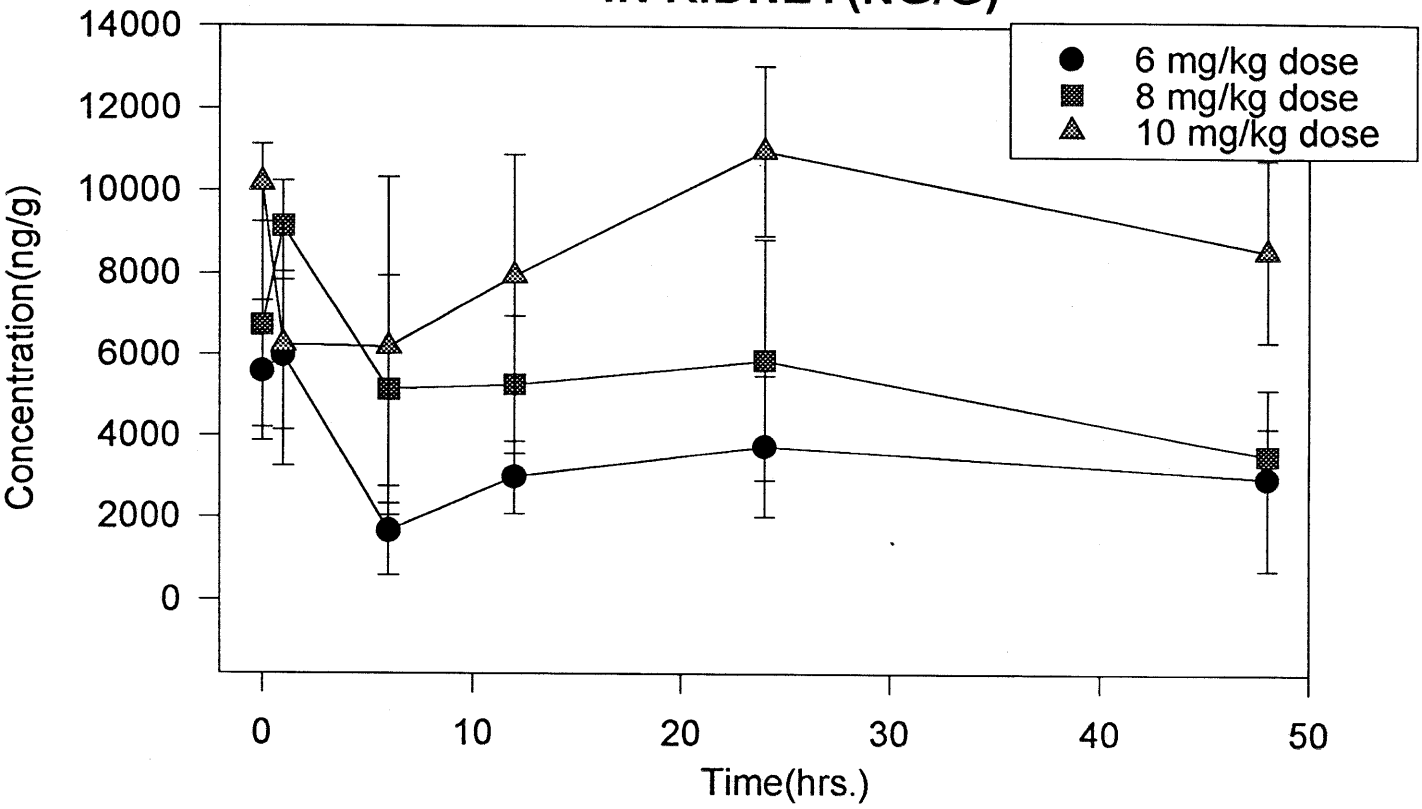


FIGURE 67. Terfenadine concentration in kidney (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

TERFENADINE CONCENTRATION IN KIDNEY(NG/G)



TERFENADINE CONCENTRATION IN KIDNEY(NG/G)REGRESSION ORDER:2

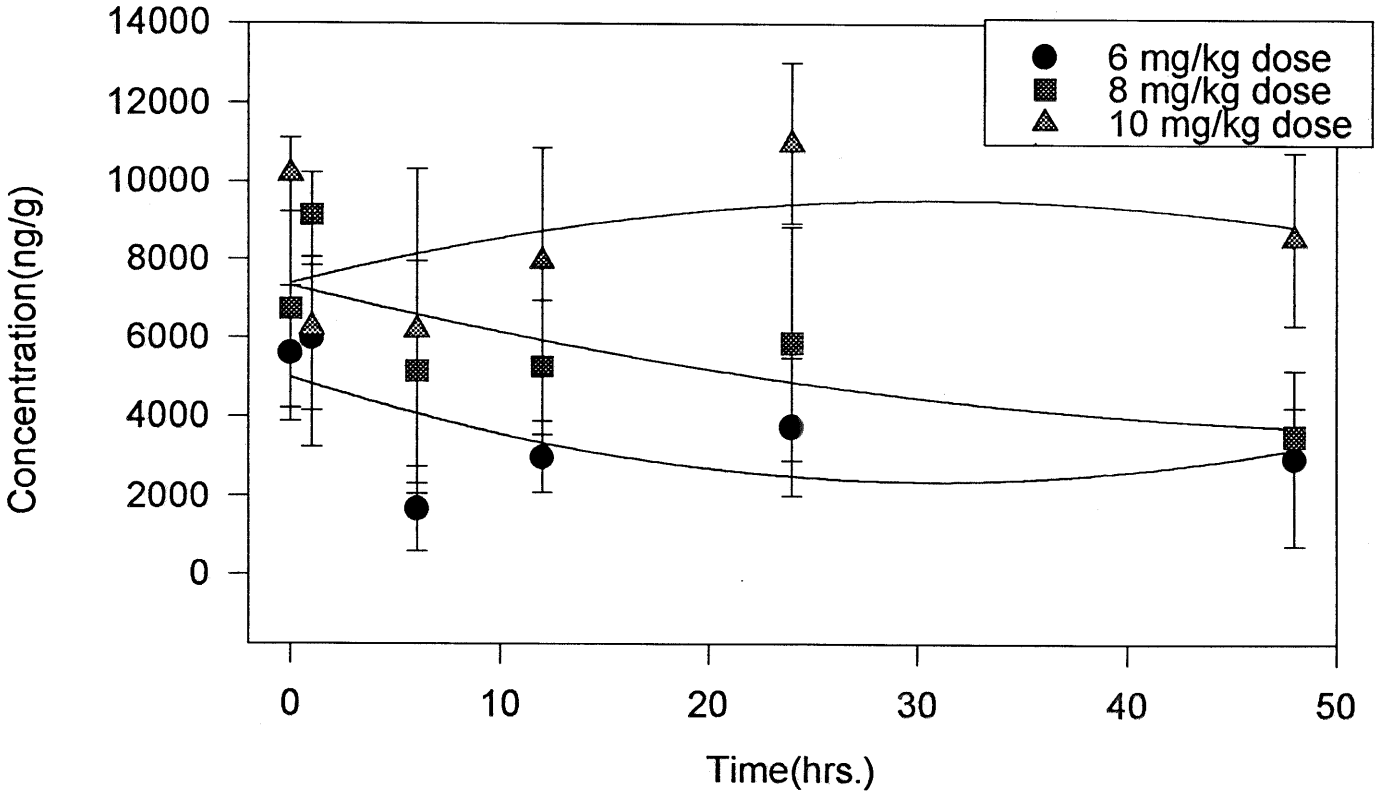
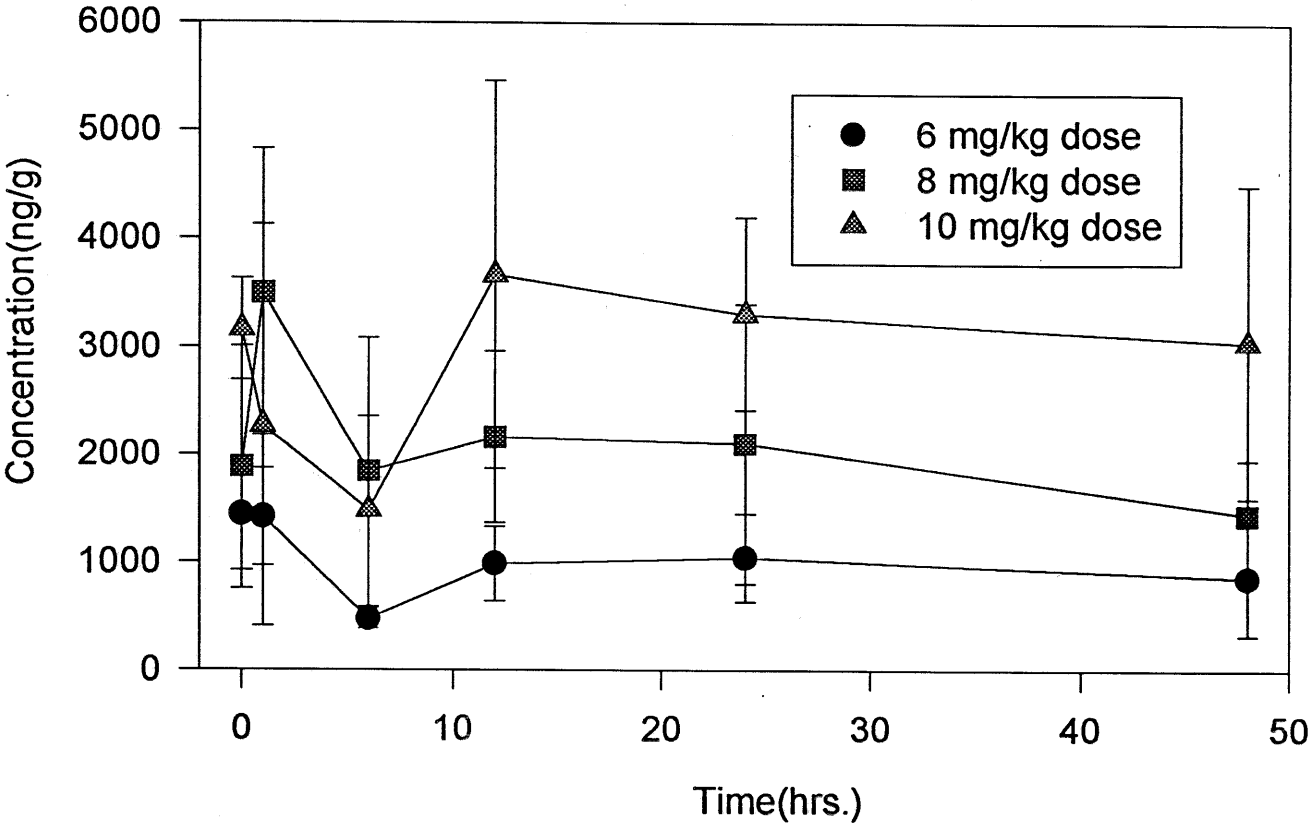


FIGURE 68. Terfenadine concentration in liver (ng/g, mean ± S.D., n = 3) at each time point: upper graph is a point-to-point analysis and lower graph is a second-order regression analysis

(figure on next page)

TERFENADINE CONCENTRATION IN LIVER(NG/G)



TERFENADINE CONCENTRATION IN LIVER(NG/G)REGRESSION ORDER:2

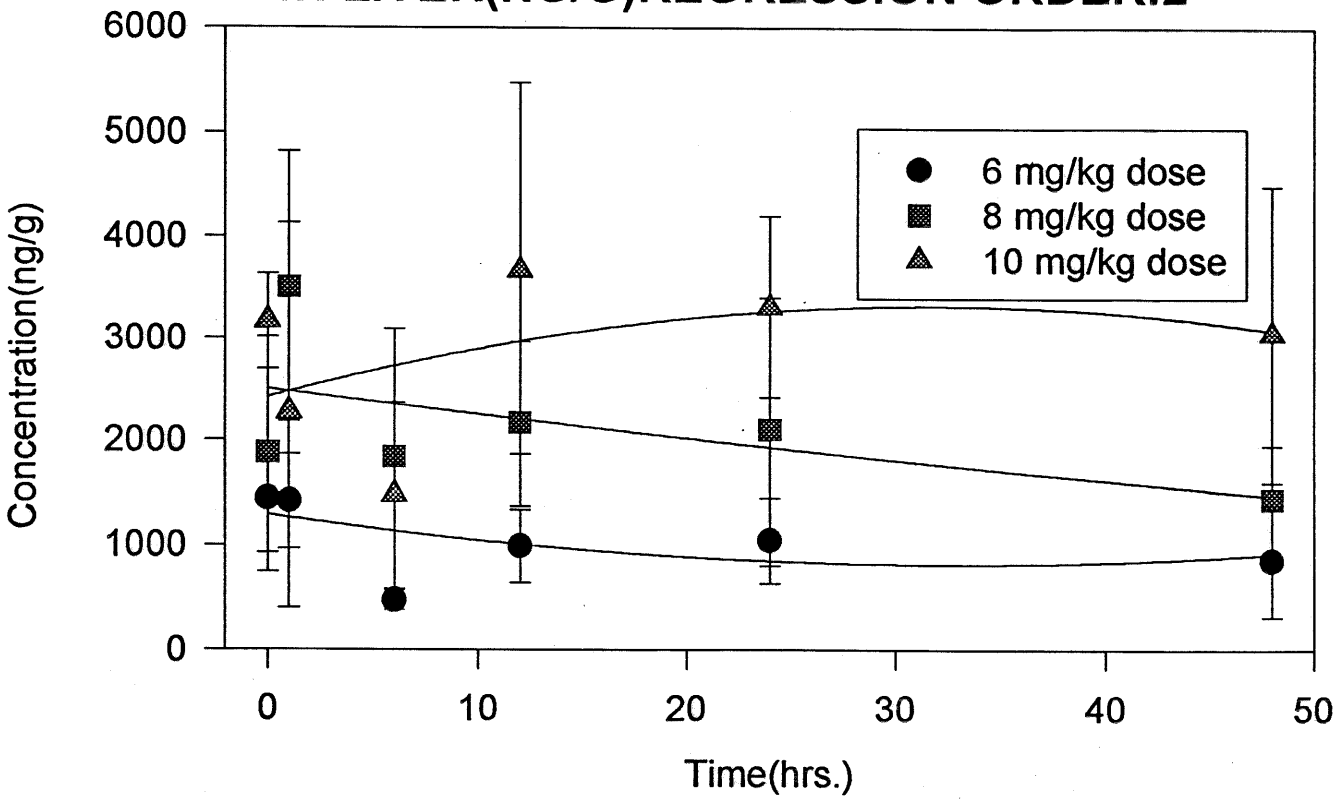
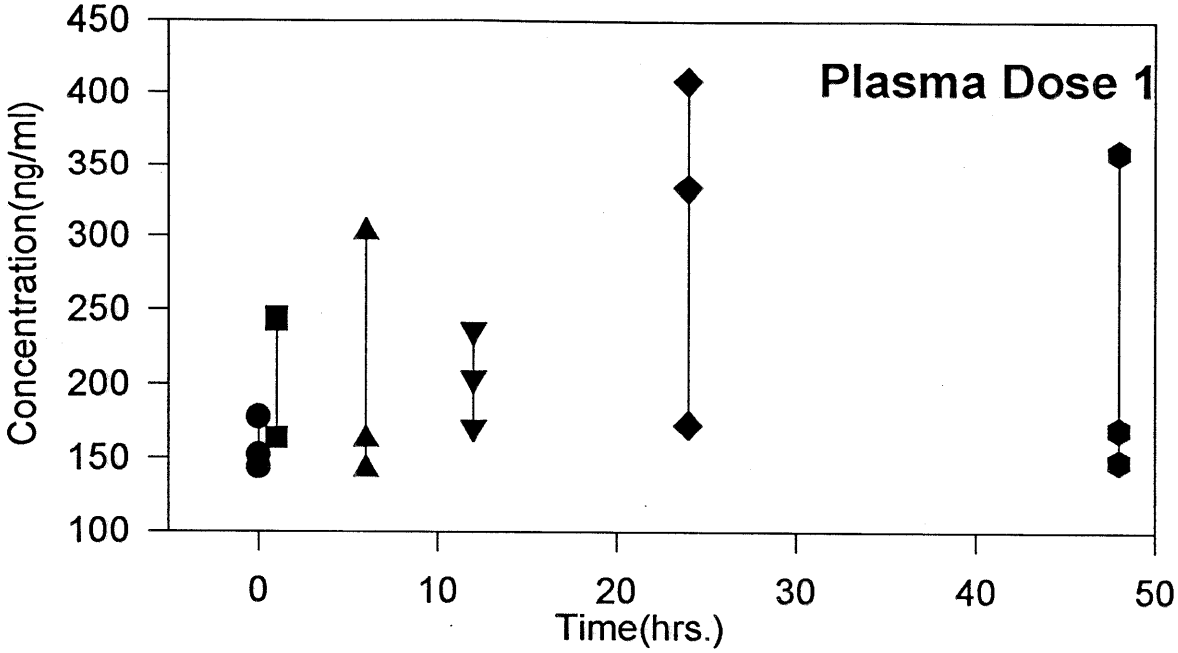
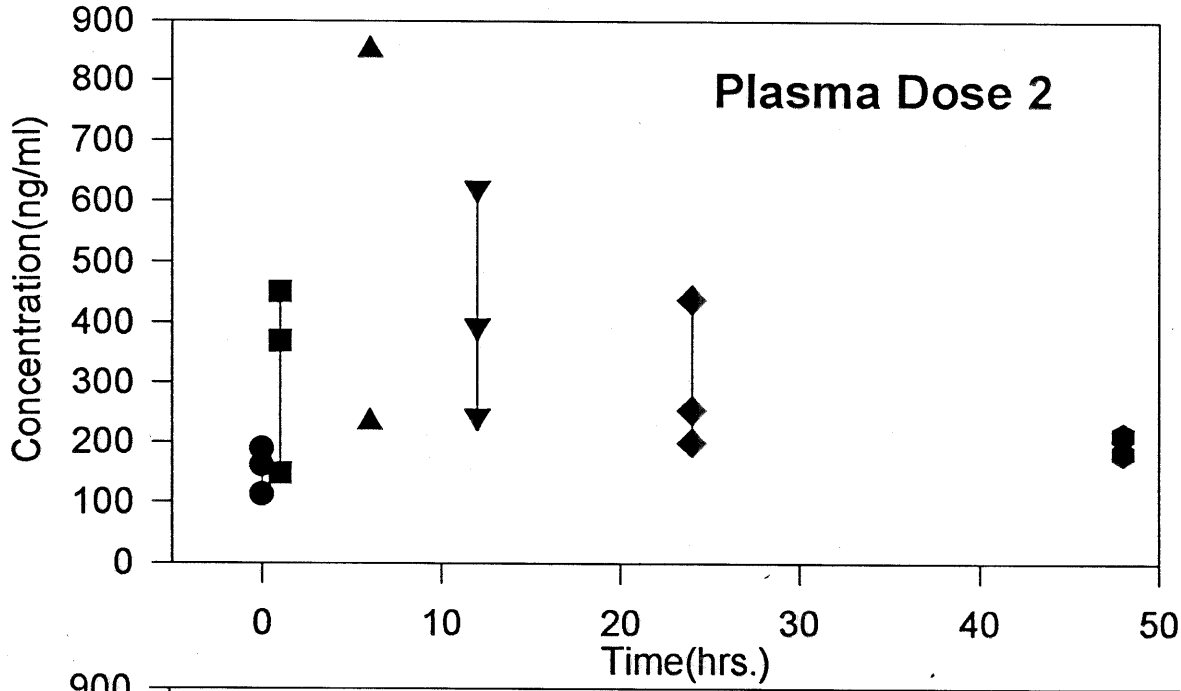


FIGURE 69. Plasma data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three terfenadine dosages (dose 1 = 6 mg/kg, dose 2 = 8 mg/kg, and dose 3 = 10 mg/kg)
(figure on next page)


Plasma Dose 1
Concentration(ng/ml)
450
400
350
300
250
200
150
100
0
10
20
30
40
50
Time(hrs.)


Plasma Dose 2
Concentration(ng/ml)
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
Time(hrs.)

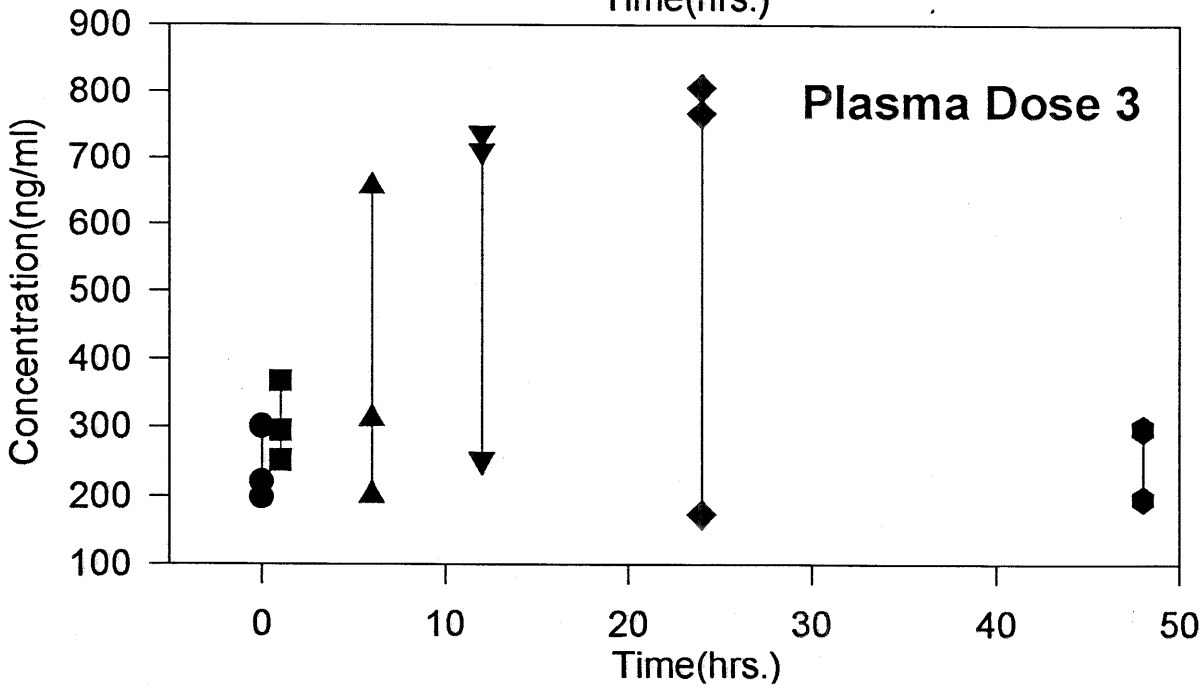
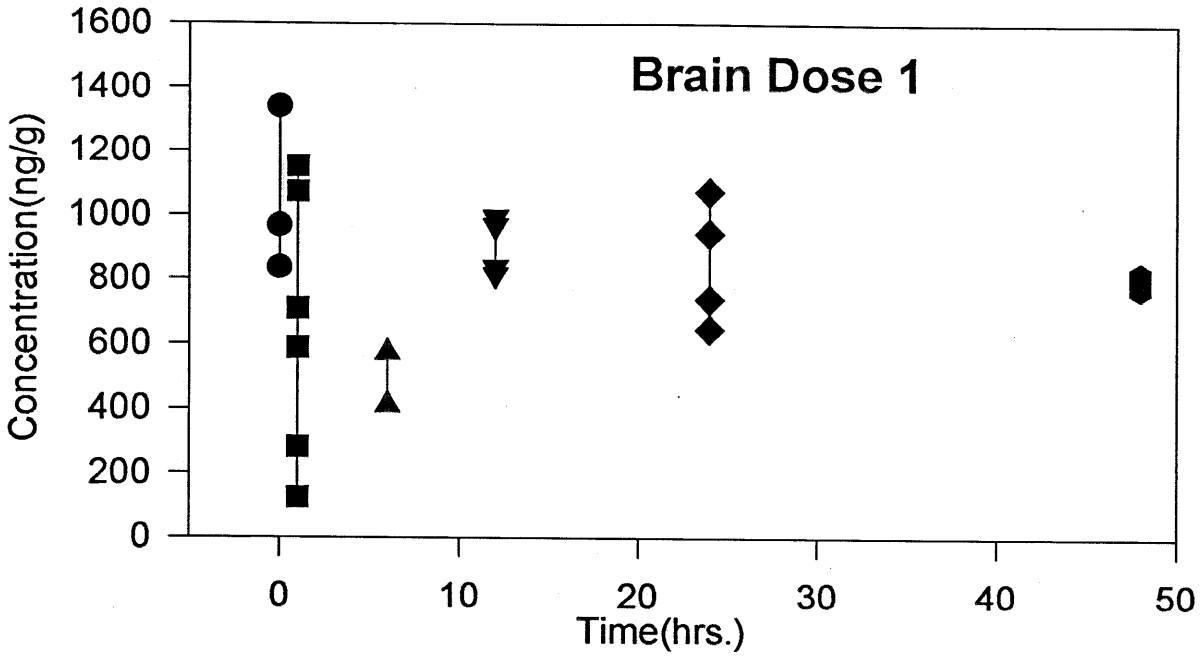
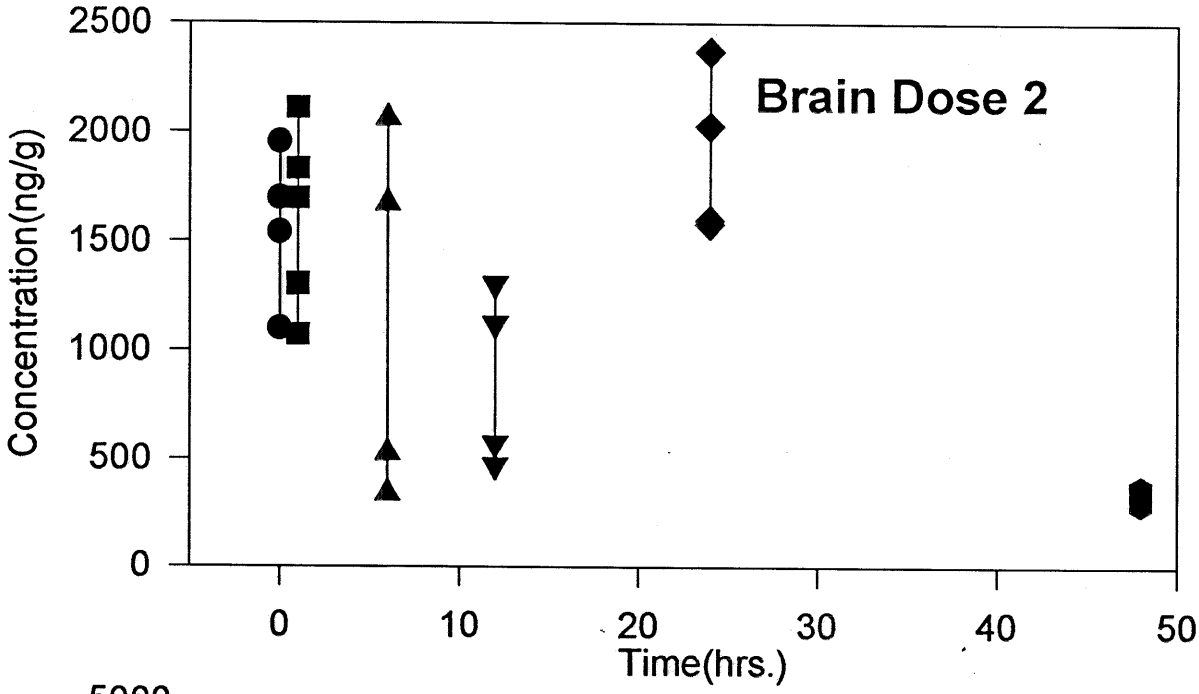

Plasma Dose 3
Concentration(ng/ml)
900
800
700
600
500
400
300
200
100
0
10
20
30
40
50
Time(hrs.)

FIGURE 70. Brain data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three terfenadine dosages (dose 1 = 6 mg/kg, dose 2 = 8 mg/kg, and dose 3 = 10 mg/kg)
(figure on next page)


Brain Dose 1
Concentration(ng/g)
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Time(hrs.)


Brain Dose 2
Concentration(ng/g)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Time(hrs.)

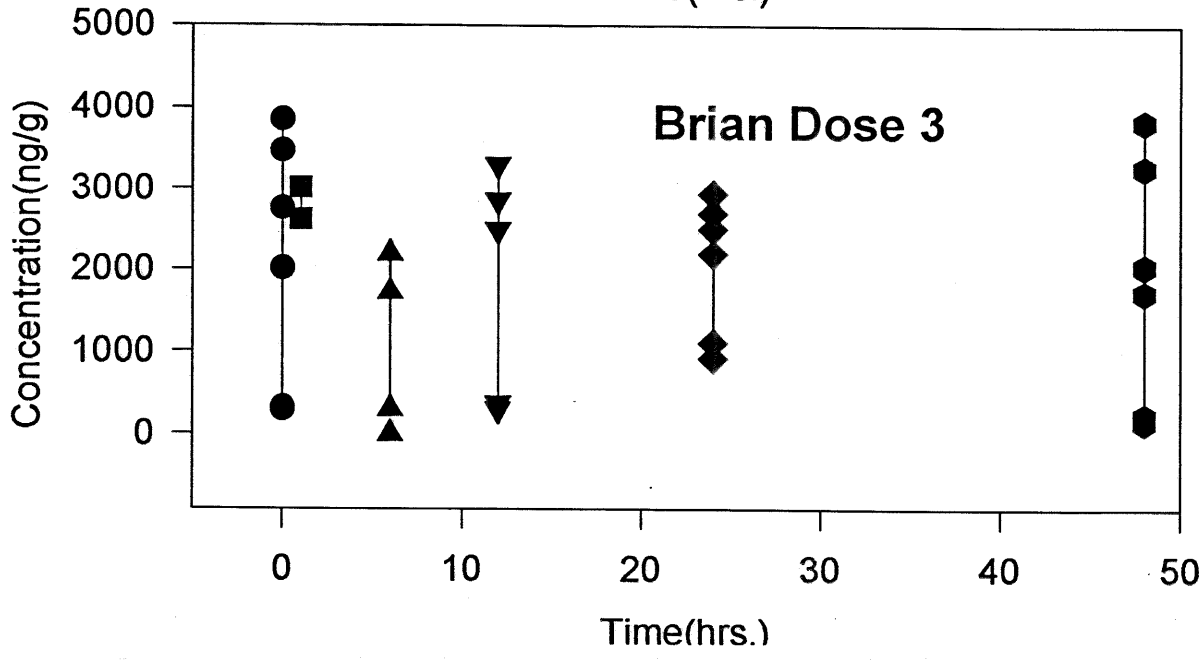
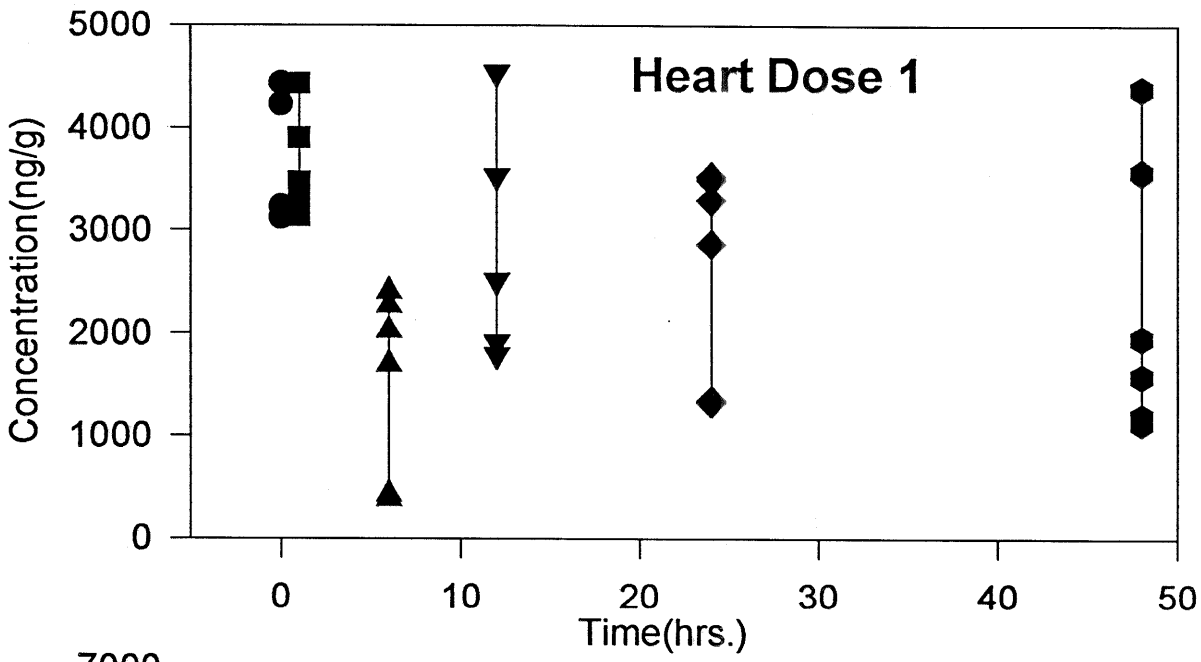
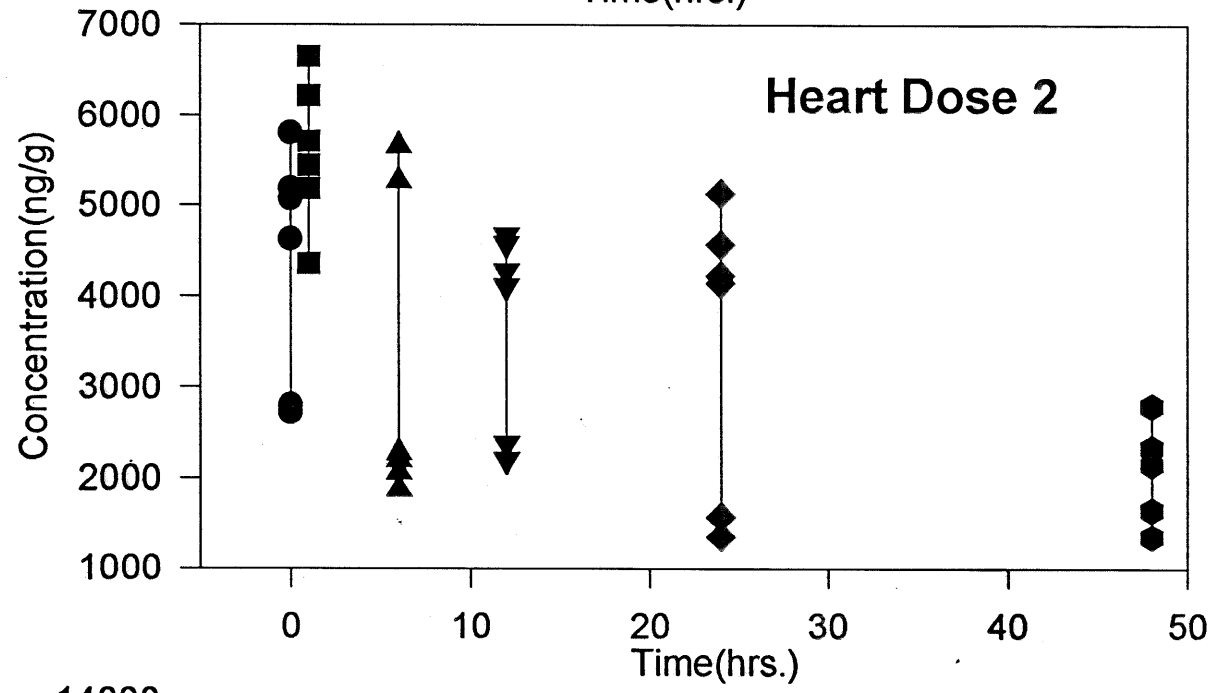

Brian Dose 3
Concentration(ng/g)
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 71. Heart data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three terfenadine dosages (dose 1 = 6 mg/kg, dose 2 = 8 mg/kg, and dose 3 = 10 mg/kg)
(figure on next page)


Heart Dose 1
Concentration(ng/g)
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)


Heart Dose 2
Concentration(ng/g)
7000
6000
5000
4000
3000
2000
1000
0
10
20
30
40
50
Time(hrs.)

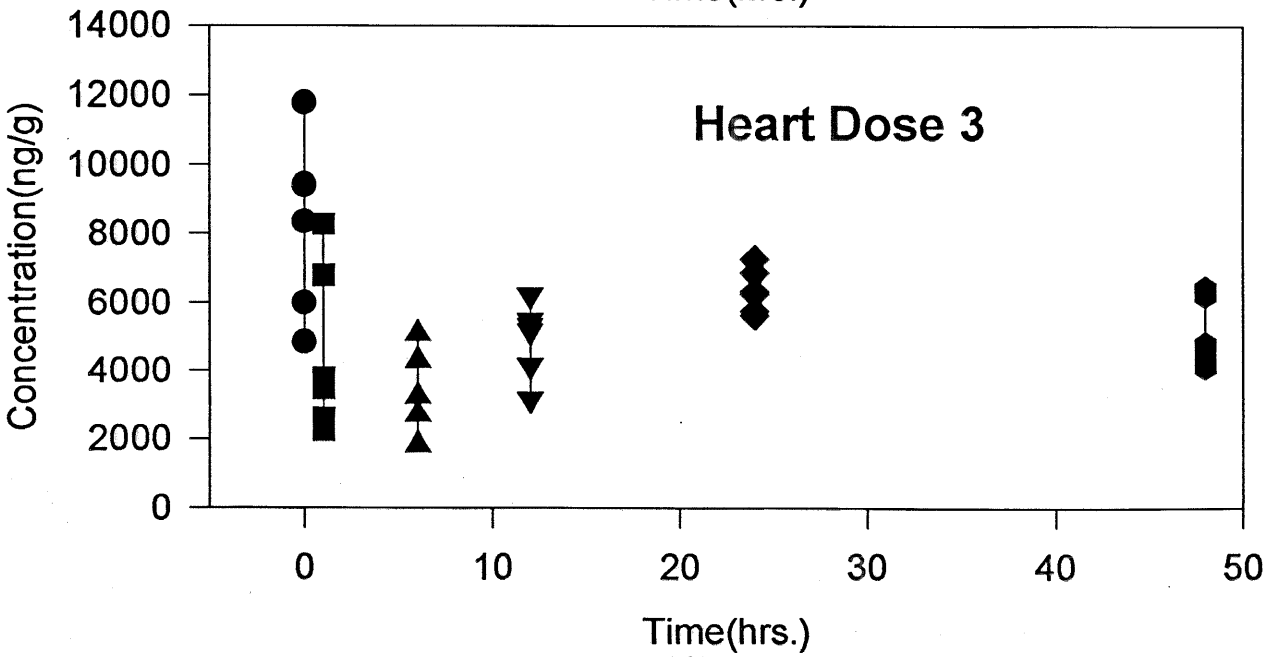
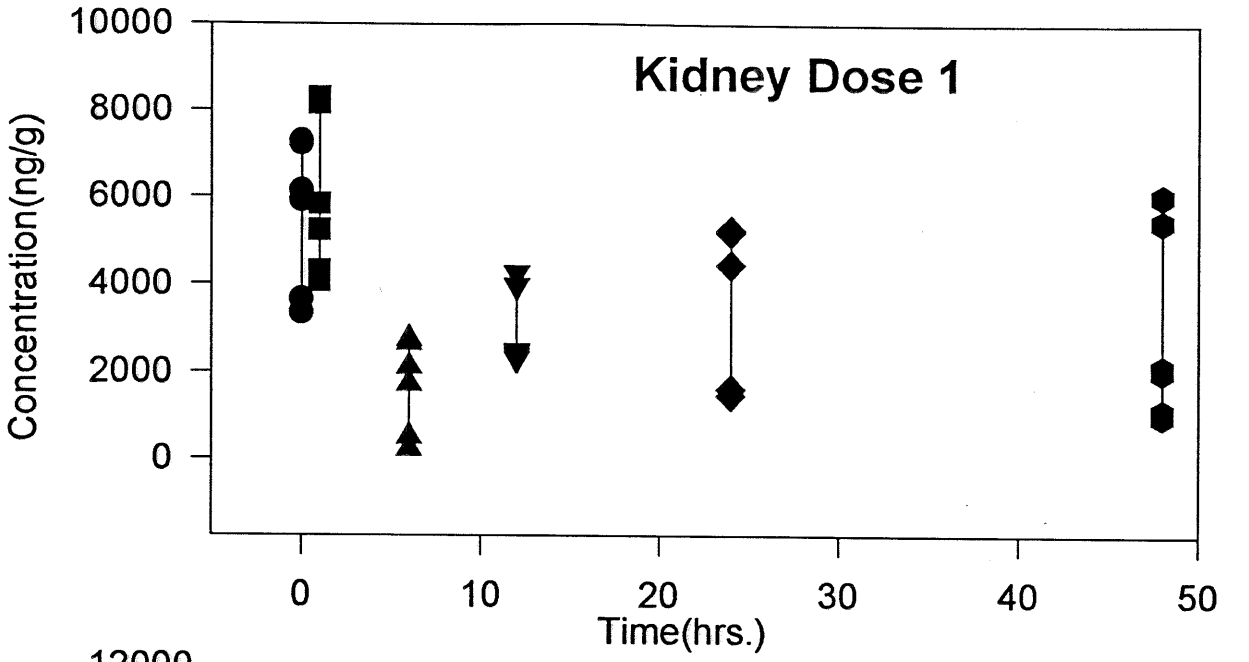
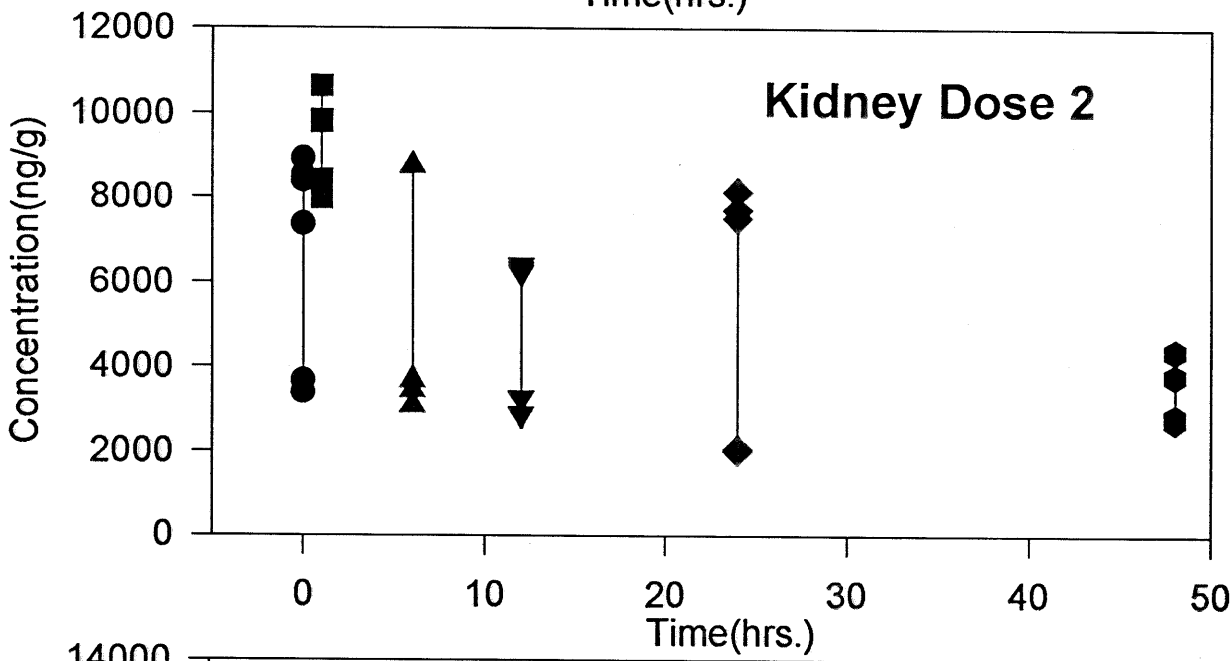

Heart Dose 3
Concentration(ng/g)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 72. Kidney data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three terfenadine dosages (dose 1 = 6 mg/kg, dose 2 = 8 mg/kg, and dose 3 = 10 mg/kg)
(figure on next page)


Kidney Dose 1
Concentration(ng/g)
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)


Kidney Dose 2
Concentration(ng/g)
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

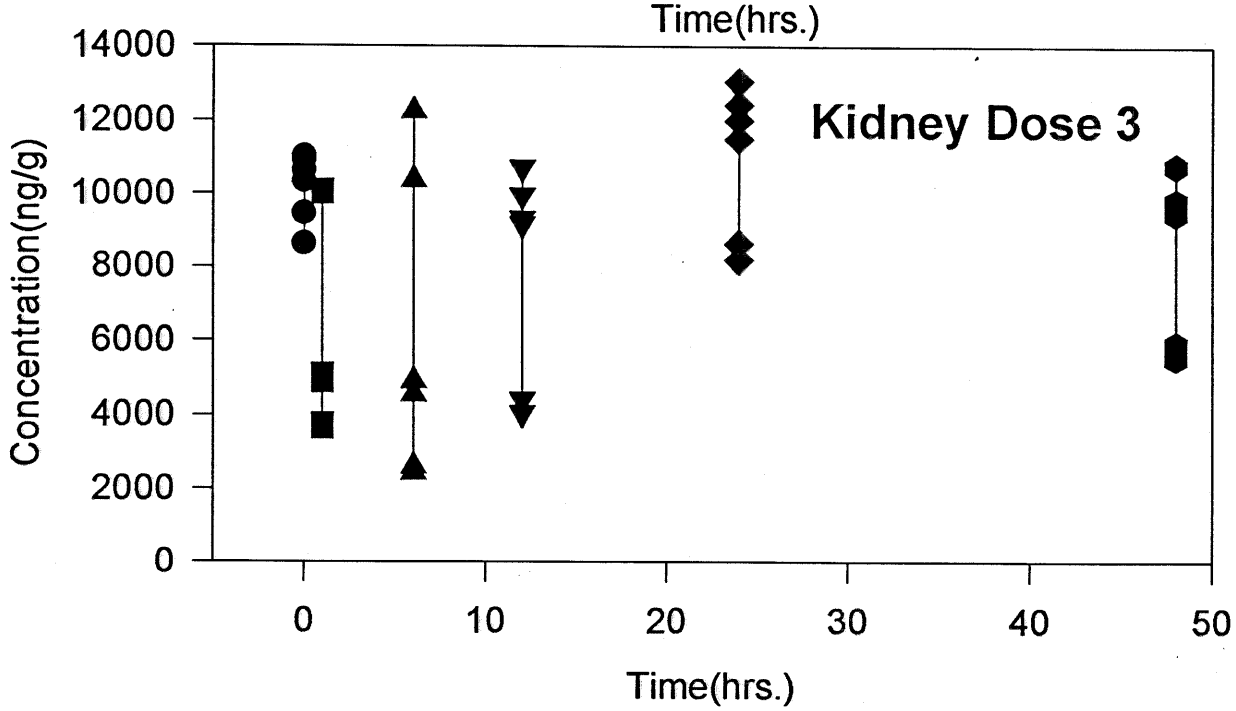
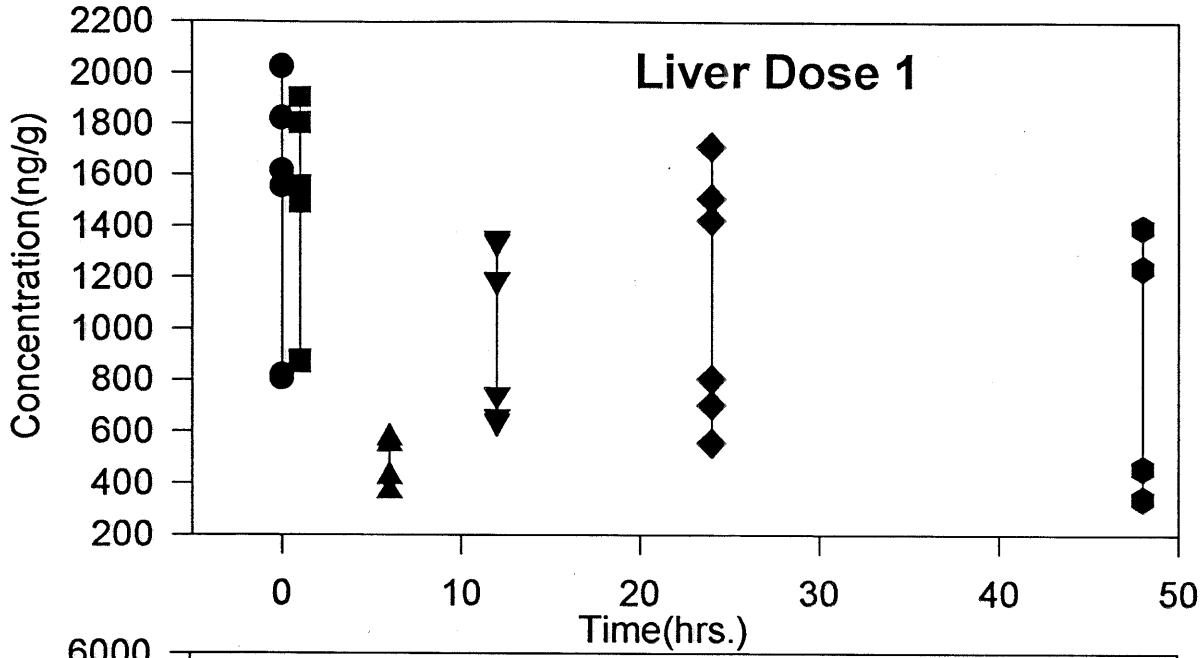
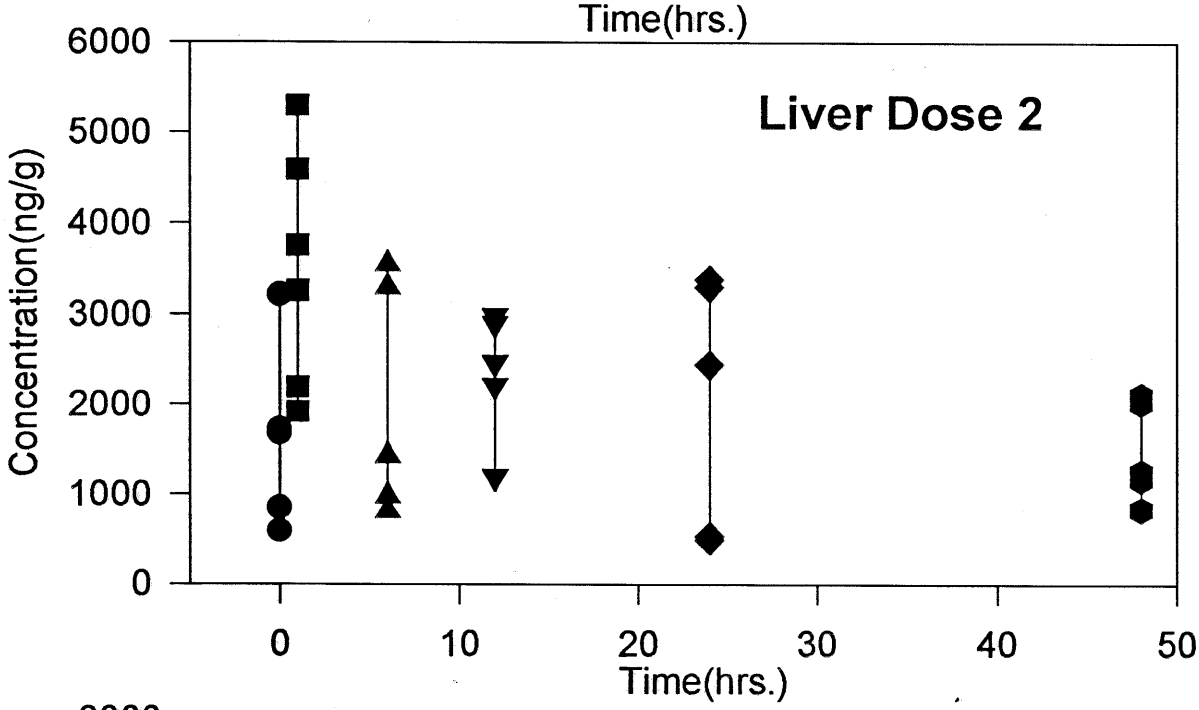
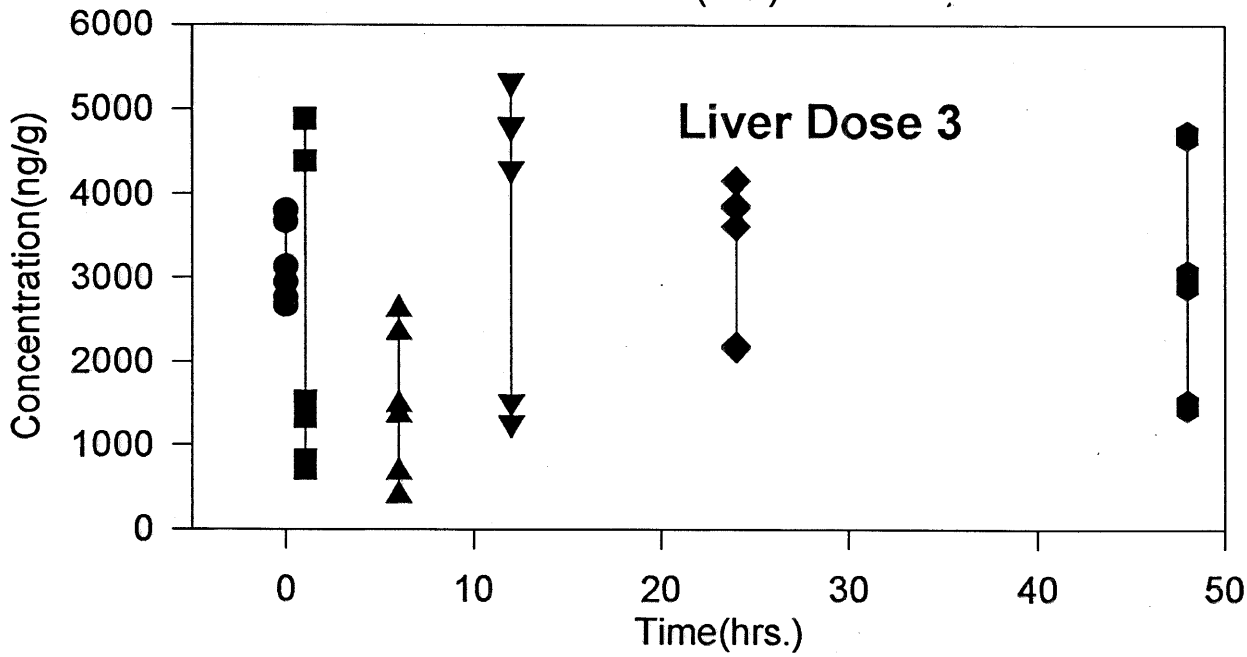

Kidney Dose 3
Concentration(ng/g)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
Time(hrs.)

FIGURE 73. Liver data from each time point at 0, 1, 6, 12, 24, and 48 hours for the three terfenadine dosages (dose 1 = 6 mg/kg, dose 2 = 8 mg/kg, and dose 3 = 10 mg/kg) (figure on next page)


Liver Dose 1
Concentration(ng/g)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10
20
30
40
50
Time(hrs.)


Liver Dose 2
Concentration(ng/g)
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)


Liver Dose 3
Concentration(ng/g)
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Time(hrs.)

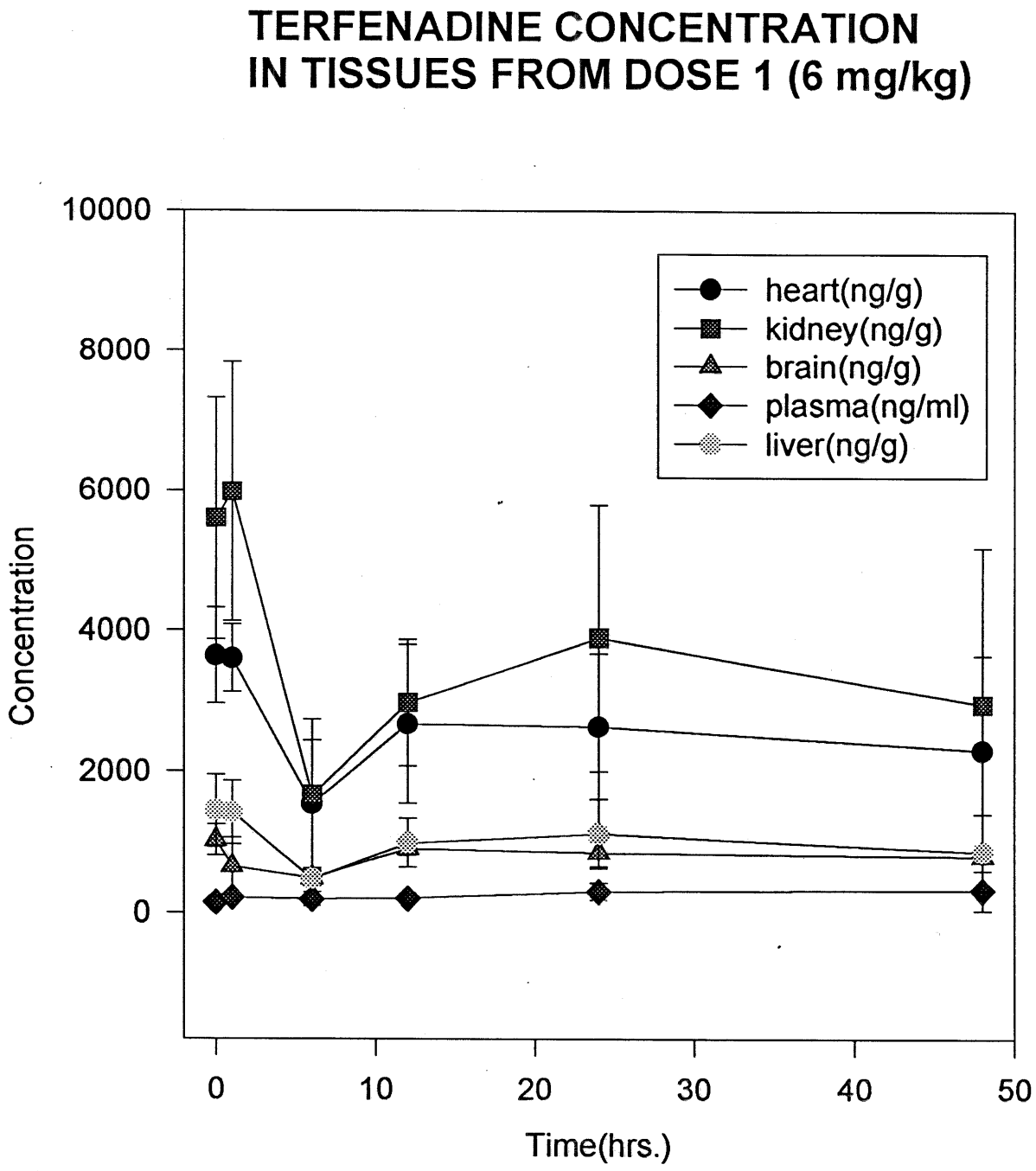


FIGURE 74. Point-to-point analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 6 mg/kg dose

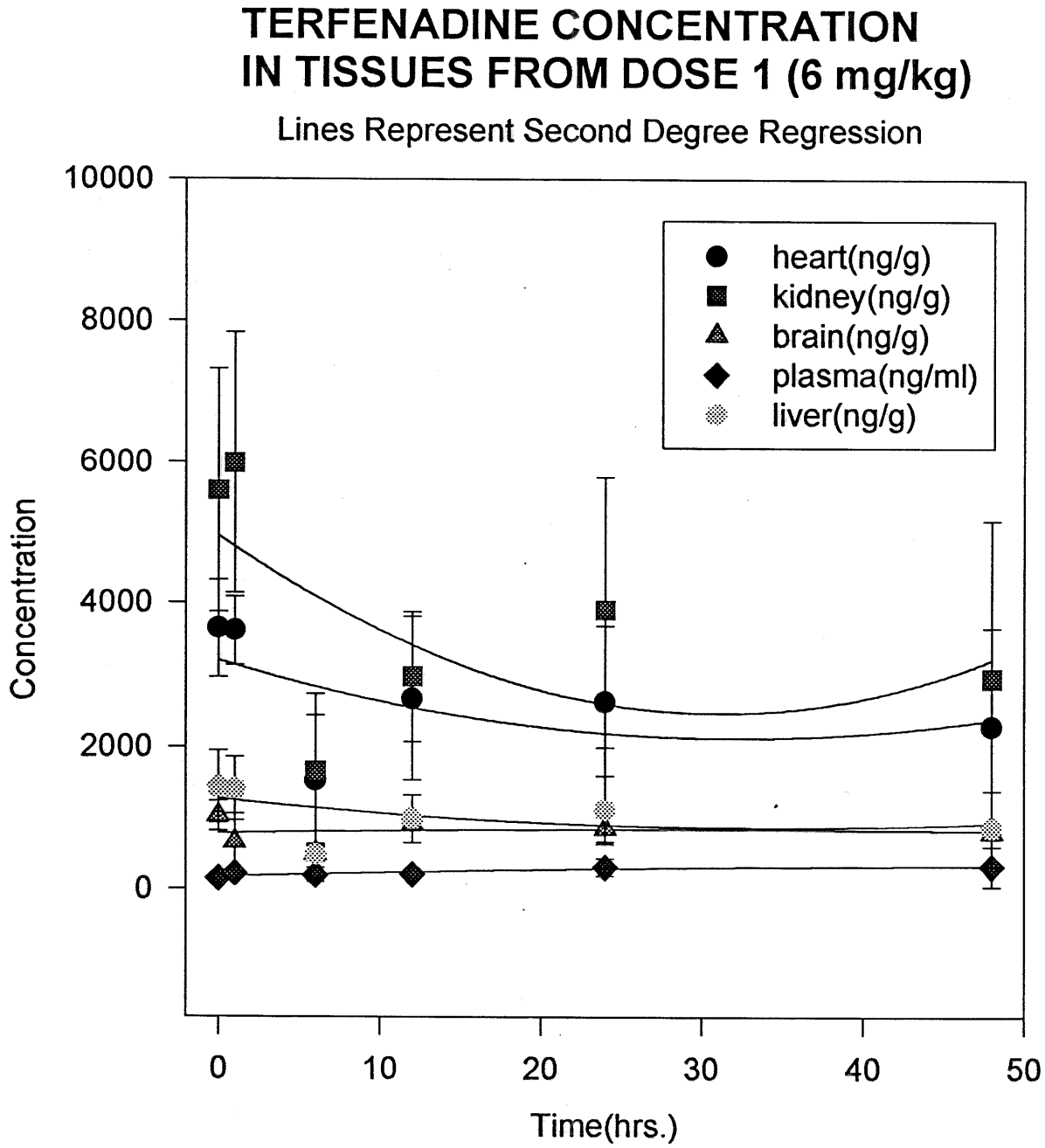


FIGURE 75. Second-order regression analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 6 mg/kg dose

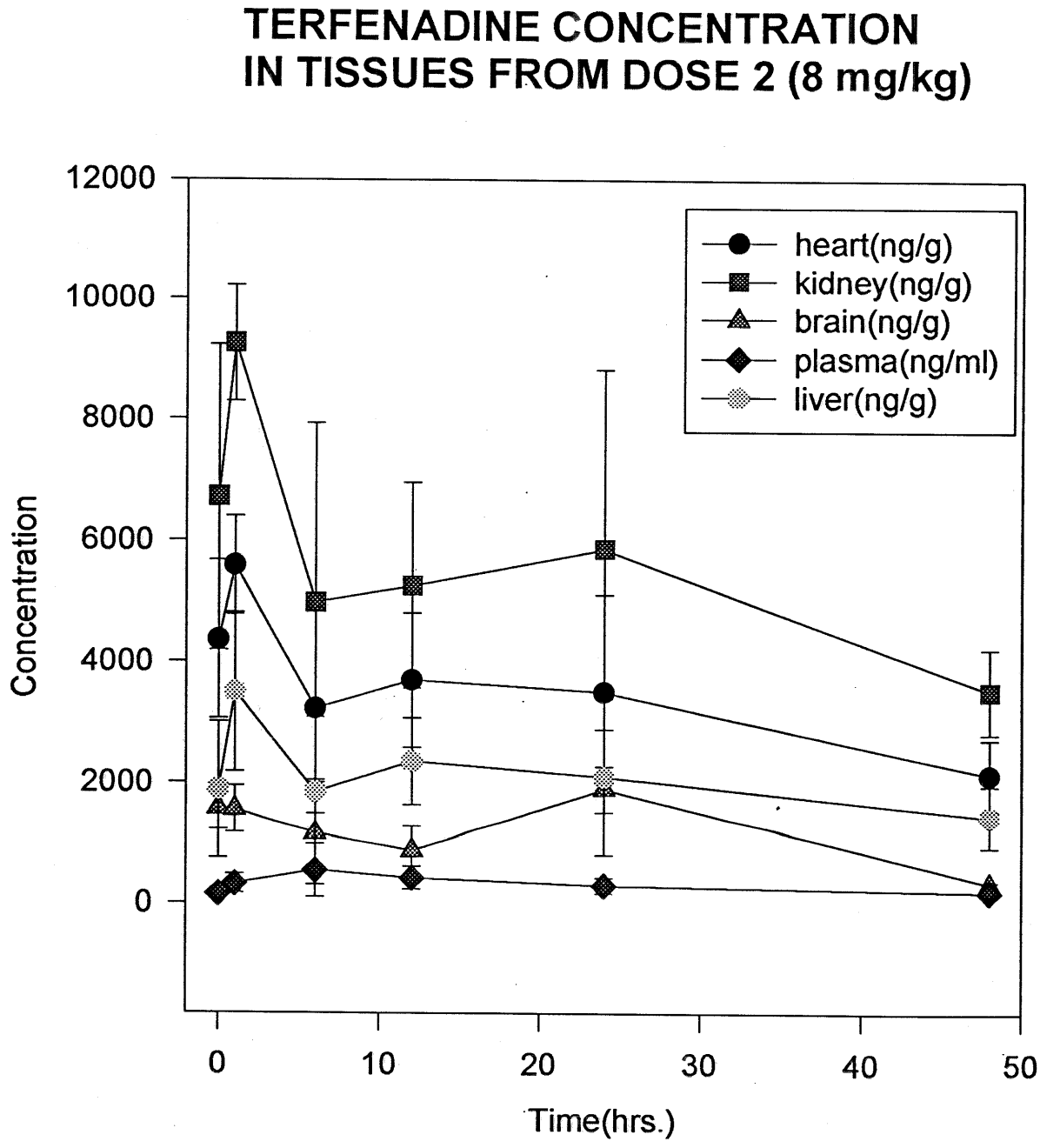


FIGURE 76. Point-to-point analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 8 mg/kg dose

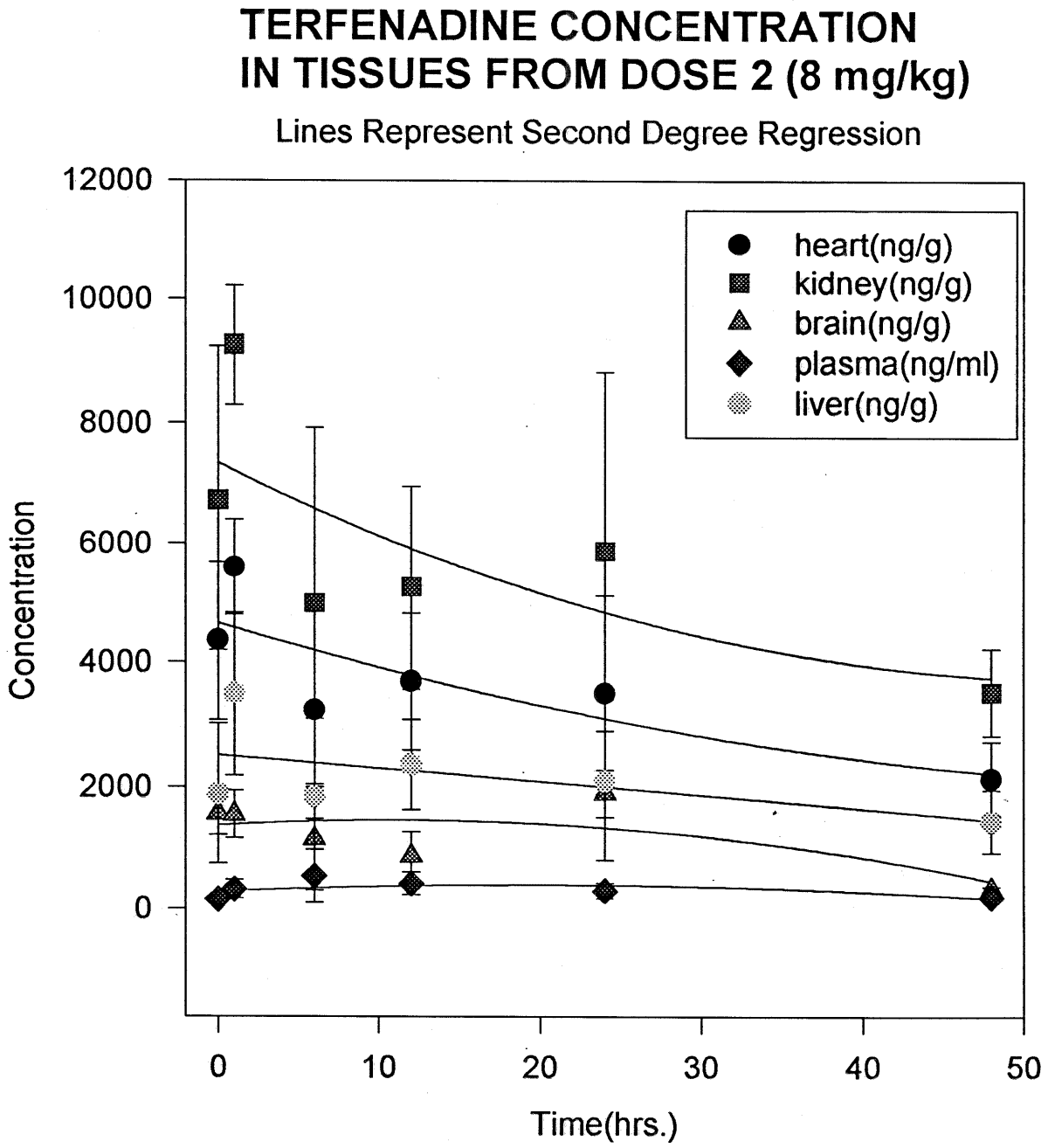


FIGURE 77 Second-order regression analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 8 mg/kg dose

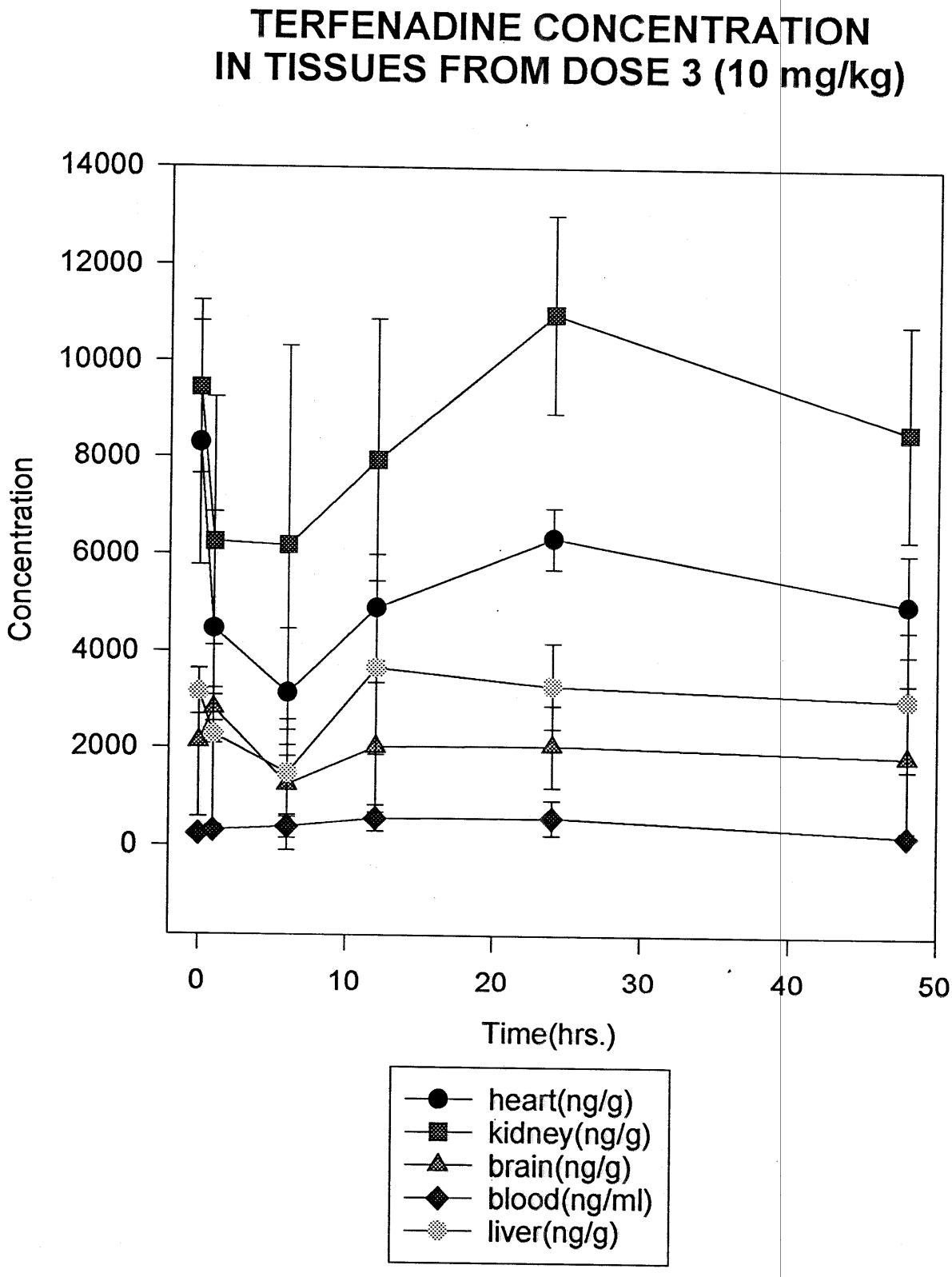


FIGURE 78. Point-to-point analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 10 mg/kg dose

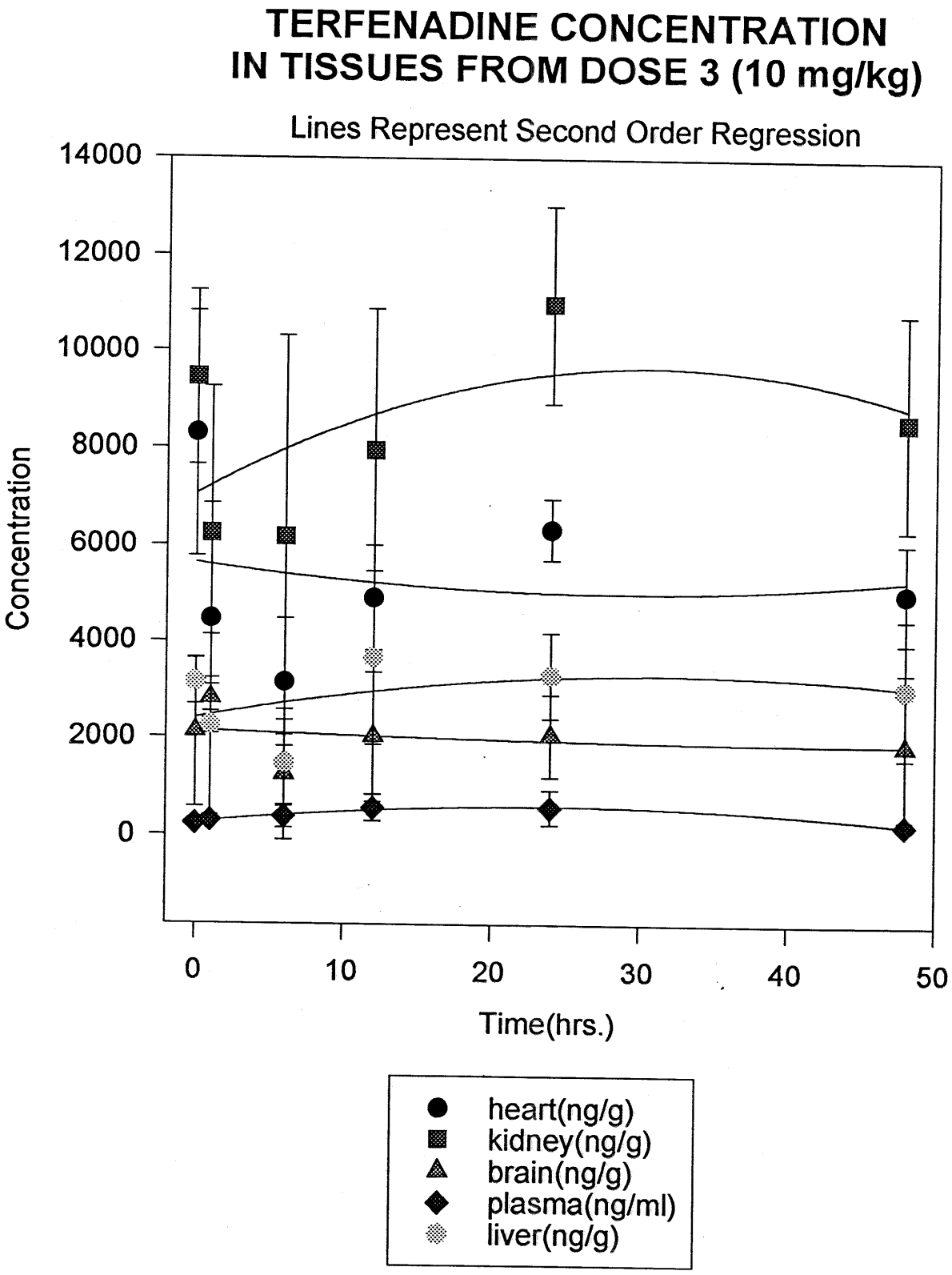


FIGURE 79. Second-order regression analysis of terfenadine concentration (mean ± S.D., n = 3) in heart, kidney, brain, plasma and liver from 10 mg/kg dose

APPENDIX

ALPRAZOLAM

Concentration in tissue/plasma (ng/g or ng/mL, +/- s.d.)

	Hrs	Dose 1		Dose 2		Dose 3	
Heart	0	1650.20	(62.29)	1569.98	(202.20)	4862.78	(2215.03)
	1	951.69	(118.00)	2205.48	(211.83)	2241.10	(391.56)
	6	1942.63	(410.58)	1853.60	(230.36)	4402.68	(1106.38)
	12	1543.07	(191.66)	3304.23	(617.59)	2098.49	(982.05)
	24	1000.34	(191.66)	1123.27	(106.11)	1945.32	(365.54)
	48	1370.62	(502.37)	3434.15	(1191.54)	3032.79	(343.02)
Kidney	0	1601.93	(140.21)	1234.90	(156.82)	3049.80	(1556.76)
	1	1115.90	(265.06)	1145.95	(251.16)	1897.45	(396.28)
	6	851.04	(544.90)	1641.06	(104.49)	2852.81	(1641.35)
	12	1079.80	(482.38)	2031.11	(936.26)	2201.79	(1294.88)
	24	656.51	(446.49)	1341.50	(643.25)	1239.20	(185.93)
	48	813.51	(251.28)	1273.86	(571.41)	1853.76	(456.44)
Brain	0	876.06	(90.80)	968.08	(288.51)	2659.95	(1641.04)
	1	643.32	(181.40)	1321.53	(130.95)	2048.97	(158.00)
	6	592.81	(349.12)	1167.87	(266.72)	2083.74	(1217.08)
	12	692.60	(331.45)	936.22	(593.84)	1303.95	(386.72)
	24	273.24	(182.73)	694.90	(125.25)	838.50	(129.75)
	48	466.64	(98.99)	1189.15	(171.75)	1222.34	(422.48)
Liver	0	1805.01	(110.05)	1934.37	(786.75)	4761.49	(3110.27)
	1	1431.77	(143.52)	2510.49	(71.17)	3164.45	(578.96)
	6	1423.88	(248.02)	2361.37	(361.70)	4106.02	(2128.76)
	12	1447.71	(525.50)	1573.78	(818.83)	1868.55	(655.20)
	24	890.12	(218.39)	1599.86	(141.10)	1841.71	(249.33)
	48	1683.84	(169.60)	3155.06	(562.04)	2847.33	(789.31)
Blood	0	644.11	(134.60)	886.38	(299.32)	1474.36	(946.48)
	1	626.82	(199.95)	973.35	(29.15)	1625.64	(242.59)
	6	636.12	(467.58)	744.95	(79.31)	1521.83	(464.87)
	12	827.04	(857.25)	591.84	(209.96)	884.91	(227.77)
	24	280.11	(55.67)	676.00	(334.85)	692.66	(173.47)
	48	256.71	(32.32)	761.25	(365.42)	638.53	(252.16)

ATENOLOL

Concentration in tissue/plasma (ng/g or ng/mL, +/- s.d.)

	Hrs.	Dose 1		Dose 2		Dose 3	
Heart	0	3352.29	(768.73)	3123.26	(1315.34)	5168.49	(3369.69)
	1	6836.30	(3329.00)	2536.30	(1771.61)	1831.08	(580.65)
	6	4199.43	(2098.00)	3086.74	(1522.34)	3693.31	(3219.43)
	12	3903.66	(2670.03)	4270.10	(665.20)	7311.65	(1346.75)
	24	6371.44	(2878.76)	5799.82	(2621.13)	11597	(1055.50)
	48	5823.10	(2815.40)	6605.45	(948.29)	4686.59	(4106.14)
Kidney	0	18879	(8957.40)	13301	(4509.01)	15643	(2779.70)
	1	14417	(5626.38)	8922.85	(366.94)	13038	(7200.99)
	6	20862	(6755.29)	18212	(4380.14)	24056	(16297)
	12	11969	(5190.92)	15747	(4306.66)	13541	(3233.30)
	24	28256	(9391.46)	12501	(5251.67)	21654	(4111.22)
	48	10938	(5984.29)	25759	(22718)	11524	(8890.76)
Brain	0	102.62	(79.94)	97.68	(51.76)	139.49	(86.66)
	1	207.76	(155.81)	120.39	(64.46)	72.39	(34.43)
	6	352.71	(205.98)	127.27	(98.27)	181.36	(165.64)
	12	217.07	(177.80)	434.12	(28.26)	686.22	(291.61)
	24	1592.49	(788.81)	846.78	(421.33)	1047.84	(446.89)
	48	1197.27	(544.70)	1478.68	(496.09)	1747.84	(1164.70)
Liver	0	5164.09	(694.32)	4132.74	(650.63)	5692.95	(2961.15)
	1	4577.72	(2099.41)	4346.13	(1989.17)	2782.34	(586.60)
	6	6465.41	(2469.17)	3867.37	(787.86)	4792.49	(1739.50)
	12	3371.30	(2147.85)	4861.98	(1243.76)	6181.51	(798.86)
	24	5589.98	(2159.09)	6820.80	(2484.95)	9739.28	(1473.92)
	48	5029.51	(3133.69)	6388.00	(1071.65)	5196.12	(4968.82)
Plasma	0	2725.76	(237.60)	2827.27	(515.89)	3344.01	(801.73)
	1	3960.33	(2005.05)	2533.40	(267.93)	2270.87	(458.46)
	6	2646.09	(1752.36)	3026.27	(817.07)	4577.31	(2698.21)
	12	2212.33	(36.76)	4778.39	(965.81)	6831.34	(1902.61)
	24	5939.44	(3063.17)	6403.77	(2952.16)	11239.00	(1166.25)
	48	5692.82	(3676.05)	8274.62	(161.97)	5809.80	(4589.86)

TERFENADINE

Concentration in tissue/plasma (ng/g or ng/mL, +/- s.d.)

	Hrs.	Dose 1		Dose 2		Dose 3	
Heart	0	3627.09	(650.72)	4371.67	(1303.04)	8301.40	(2524.66)
	1	3608.24	(477.49)	5593.11	(803.39)	4521.05	(2442.31)
	6	1532.65	(905.49)	3226.21	(1745.75)	3150.41	(1328.41)
	12	2676.06	(1130.66)	3701.75	(1113.99)	4913.62	(1089.77)
	24	2637.85	(1037.51)	3503.67	(1616.82)	6344.40	(627.78)
	48	2297.11	(1356.52)	2185.10	(590.91)	5025.11	(1034.20)
Kidney	0	5599.23	(1724.84)	6719.66	(2517.29)	10178	(945.38)
	1	5986.20	(1848.58)	9138.23	(1090.39)	6248.52	(3008.16)
	6	1663.54	(1076.53)	5133.86	(2811.80)	6182.47	(4139.21)
	12	2974.37	(895.54)	5254.69	(1695.35)	7245.87	(2926.97)
	24	3742.74	(1741.79)	5861.90	(2969.06)	10981	(2052.76)
	48	2957.11	(2221.64)	3522.89	(706.10)	8566.43	(2226.36)
Brain	0	1028.37	(216.31)	1572.58	(358.77)	2118.34	(1536.69)
	1	653.75	(411.64)	1553.10	(391.32)	2816.68	(277.14)
	6	491.30	(113.57)	1149.60	(848.15)	2150.99	(130.18)
	12	904.16	(90.72)	867.54	(408.55)	2029.98	(1336.52)
	24	851.48	(194.49)	1895.58	(381.03)	2056.73	(851.14)
	48	788.81	(13.14)	306.58	(59.72)	1864.91	(1521.45)
Liver	0	1439.79	(513.80)	1880.57	(1128.66)	3165.91	(469.41)
	1	1416.02	(448.17)	3502.08	(1321.18)	2270.01	(1860.71)
	6	479.00	(97.24)	1837.90	(1249.46)	1473.81	(885.15)
	12	987.19	(343.22)	2162.09	(797.36)	3666.83	(1800.42)
	24	1042.94	(494.30)	2101.05	(1297.85)	3307.06	(891.28)
	48	860.60	(535.65)	1439.61	(510.42)	3044.67	(1447.59)
Plasma	0	157.81	(17.80)	154.48	(38.48)	240.25	(53.24)
	1	217.01	(46.40)	322.36	(156.58)	304.75	(58.20)
	6	202.40	(87.24)	542.06	(437.34)	389.04	(237.15)
	12	203.87	(32.75)	420.11	(190.46)	567.36	(271.94)
	24	304.64	(120.37)	297.80	(124.86)	582.54	(355.16)
	48	225.76	(115.85)	197.75	(21.03)	246.36	(71.73)